THE INNERVATION OF THE SHOULDER JOINT[1]

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TWO FIGURES

INTRODUCTION

Certain features of the gross pattern of the nerve supply of the shoulder joint have been known since the last century. Nevertheless the innervation has been studied less than that for the hip, knee and elbow, and there have been no investigations of the distribution of nerves after they enter the capsule.

Rüdinger (1857) carried out the first systematic study of the nerve supply of this joint. He found it to be derived from the axillary and suprascapular nerves. One branch of the former arose in the axilla and descended to the anteroinferior region of the capsule. Another branch arose from the axillary nerve as it began its circumflex course and supplied the posteroinferior region of the capsule. The latter distribution was especially pronounced when the inferior branch of the suprascapular nerve was lacking. He observed one case in which a muscle bundle arose from the coracoid process and anterior aspect of the capsule. A branch from the brachial plexus supplied this muscle and also gave a twig to the superior region of the capsule. He described superior and inferior branches from the suprascapular nerve. The superior one coursed laterally in the supraspinous fossa, gave a twig to the coracoromial ligament and then split into 3

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or 4 branches which entered the capsule deep to the tendon of the supraspinatus muscle. Whether some reached the acromioclavicular joint was uncertain. The inferior articular branch arose just as the suprascapular nerve entered the infraspinous fossa. It ran laterally to the capsule where it split into several smaller branches. Some of these reached the area supplied by the lower articular branch of the axillary nerve, but no anastomoses were seen.

Fick ('04) stated that the nerves arise from the adjacent muscle nerves, that is, the axillary, suprascapular and subscapular. In Quain's Elements of Anatomy ('09) articular branches of the axillary and suprascapular nerves are described. The latter are said to arise in the supraspinous fossa, and sometimes in the infraspinous fossa. Bock and Valentin are cited to the effect that the lateral anterior thoracic nerve gives twigs to the acromioclavicular joint. Rauber is cited as describing a filament from an articular branch of the axillary nerve which ascends in the bicipital sulcus to the upper part of the humerus and the joint capsule.

The accounts in current textbooks stem from these earlier studies. Piersol's Human Anatomy ('30) describes one branch arising from the axillary nerve near its origin and another arising during its passage through the quadrilateral space. Together they supply the anterior, inferior and posterior regions of the capsule. This is similar to Rüdinger's description. A 3rd articular branch is said to ascend in the bicipital sulcus. Articular twigs from the suprascapular nerve are also mentioned.

Morris' Human Anatomy ('42), in the section on joints, states that articular twigs arise from the axillary and subscapular nerves, and also from the suprascapular nerve in both supraspinous and infraspinous fossae. In the section on the nervous system, however, no mention is made of twigs from the subscapular nerves. In Gray's Anatomy of the Human Body ('42), in the section on joints, the axillary and suprascapular nerves are given as the supply for the shoulder joint, whereas in the section on the nervous system, twigs

from the subscapular nerves are mentioned as an additional source. In Cunningham's Text-Book of Anatomy ('43), in the section on joints, the nerve supply is said to arise from the suprascapular, subscapular and axillary nerves, but in the section on the nervous system there is nothing regarding articular filaments from the subscapular nerves.

The most recent investigation appears to be that of Arkhangelsky ('31) who dissected 40 shoulder joints. According to him, the nerve supply is derived from the axillary, suprascapular and anterior thoracic nerves. He states that the axillary nerve always gives a branch about 3 cm cranial to the inferior border of the subscapularis muscle which descends to the inferior border of this muscle and there divides into twigs which are given to the anteroinferior region of the capsule. In about half the cases, he found thin branches which approached the inferolateral region of the capsule near the surgical neck of the humerus. A branch to the bicipital sulcus, as described by Rauber, was much less common. Periosteal and articular branches arise from the suprascapular nerves in both supraspinous and infraspinous fossae. Arkhangelsky dwelt at some length on the articular branches of the anterior thoracic nerves. One of these supplies the acromial process and probably gives fine twigs to the shoulder joint as well. Another branch, which occurred in about 40% of the dissections, ascends in the bicipital sulcus.

So far as could be determined, there have been no complete studies of the nerve supply of this joint in lower animals. Ghetie ('39) mentions an articular branch of the axillary nerve to the shoulder joint of the horse. Sasaoka ('38–'39) mentions a similar branch in the cat.

MATERIALS AND METHODS

Nerves were dissected in 7 adult shoulder joints so as to establish the general pattern of distribution. Four other joints were partially dissected to confirm points brought out by study of fetal joints. Four fetal joints were sectioned

serially at 10 μ and stained by a modified Masson's method.[2] Two were from a 11-week fetus and two from a 12-week fetus. All were left attached to the trunk and sectioned in a plane transverse to the trunk. The ages given are only approximate since they were determined on the basis of crown-rump measurements of fixed specimens. While the material was not satisfactory for the study of nerve endings, it was excellent for tracing nerve fibers, since even in very small bundles there were usually a few large fibers which were easily distinguished.

Diagrammatic sketches were made of representative dissections, and projection tracings were made of fetal sections which showed characteristic features of the nerve supply.

OBSERVATIONS

The major features of existing descriptions were confirmed and, in addition, branches hitherto undescribed were found. The nerve supply is derived from the suprascapular, axillary and anterior thoracic nerves, from the posterior cord of the brachial plexus, directly from the stellate and perhaps other sympathetic ganglia and possibly from the radial nerve.

In the illustrations, both of gross and microscopic material, branches are named according to their most specific origin. Thus, "suprascapular" (fig. 2a) indicates articular twigs from the suprascapular nerve.

The articular branches of the axillary nerve

Shortly after the origin of the axillary nerve from the posterior cord of the brachial plexus a small branch arises which descends laterally across the subscapularis muscle, turns around the lower edge of the muscle and divides into twigs which enter the inferior region of the capsule (fig. 1, ant.). Some of the terminal ramifications of these twigs were

[2] The first 6 dissections were done while the author was in the Department of Anatomy, Stanford University, California, 1940–1941. The fetal material was obtained through the courtesy of Prof. C. H. Danforth, Department of Anatomy, Stanford University, California.

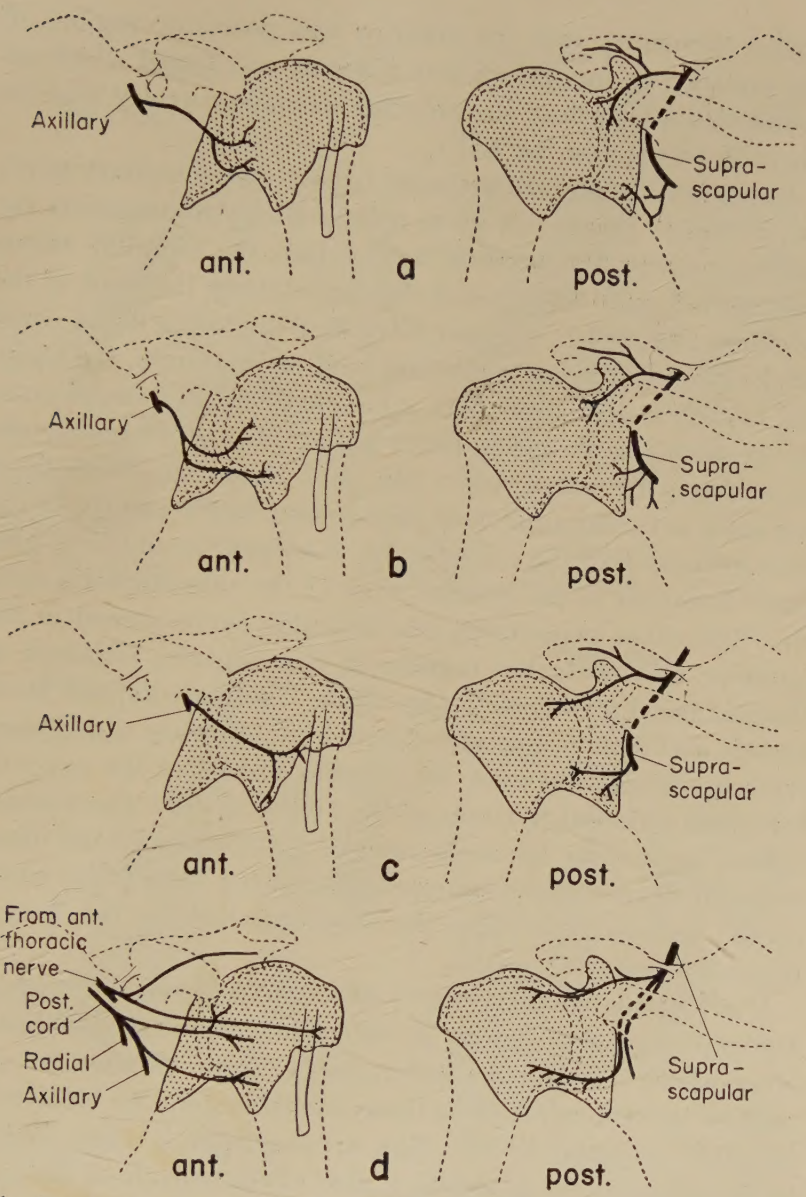

Fig. 1, a-d Anterior and posterior views of different types of distribution of nerves to the shoulder joint. The articular capsule is represented by stippling. d, ant. shows the articular branch of the lateral anterior thoracic nerve and from the posterior cord.

traced superiorly into the anterior and posterior regions of the capsule. In one case a tiny filament was found which accompanied a branch of the anterior circumflex artery into the bicipital sulcus (fig. 1c, ant.).

In the fetal joints, the articular branch of the axillary nerve has a similar course. It gives a twig which accompanies the anterior circumflex humeral artery into the bicipital sulcus and ascends until branches of this vessel enter the head of the humerus (fig. 2c-h). Beyond this point the nerve fibers could not be traced. During the initial part of its course, the bicipital branch anastomoses with a bundle of fibers derived from the posterior cord, and possibly with still another bundle derived from the radial nerve (fig. 2j). Numerous twigs from the main articular branch of the axillary nerve ramify in the inferior region of the capsule (fig. 2i-k) and, in addition, give small branches to the inferior edge of the subscapularis muscle. Still other twigs from this articular nerve ascend in the anterior and posterior regions of the capsule, adjacent to their humeral attachments (fig. 2g-i). These accompany blood vessels and both vessels and nerves give branches which penetrate the fibrous layer of the capsule and enter the synovial layer and adjacent portions of the humerus. The fibers which ascend posteriorly reach the area of distribution of the lower articular branch of the suprascapular nerve. The fibers which ascend anteriorly overlap the area supplied by the branch from the posterior cord.

Many of the small nerve bundles which enter the inferior and anteroinferior regions of the capsule course in the fibrous layer where they appear to terminate. These bundles are numerous in comparison with those which accompany blood vessels into synovial tissue. The arrangement is thus similar to that found in the posterior region of the capsule of the knee joint (Gardner, '48) and in the anteromedial region of the hip joint (Gardner, '48). The articular twigs from the posterior cord also contribute to this arrangement (see below).

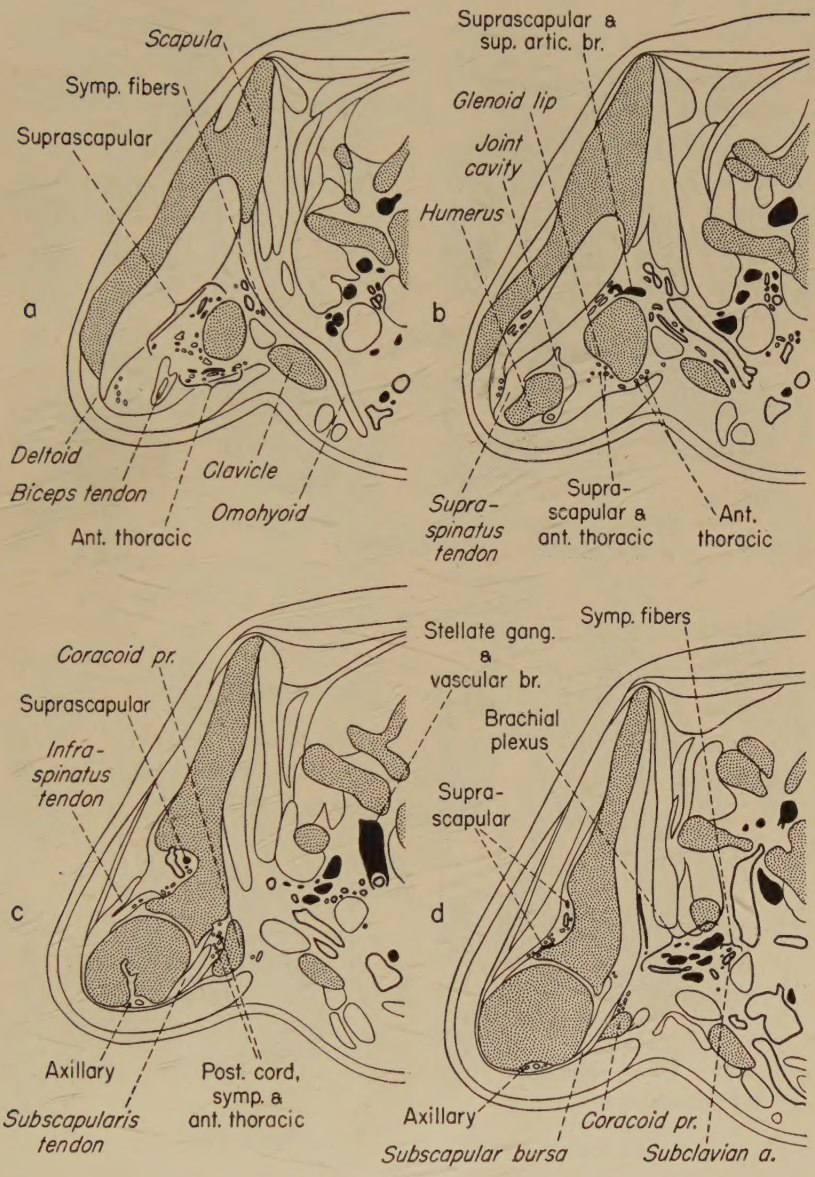

Fig. 2, a-d Projection tracings of a fetal joint (sectioned in a plane transverse to the trunk). Nerves are shown in solid black. The sections go from cranial to caudal levels.

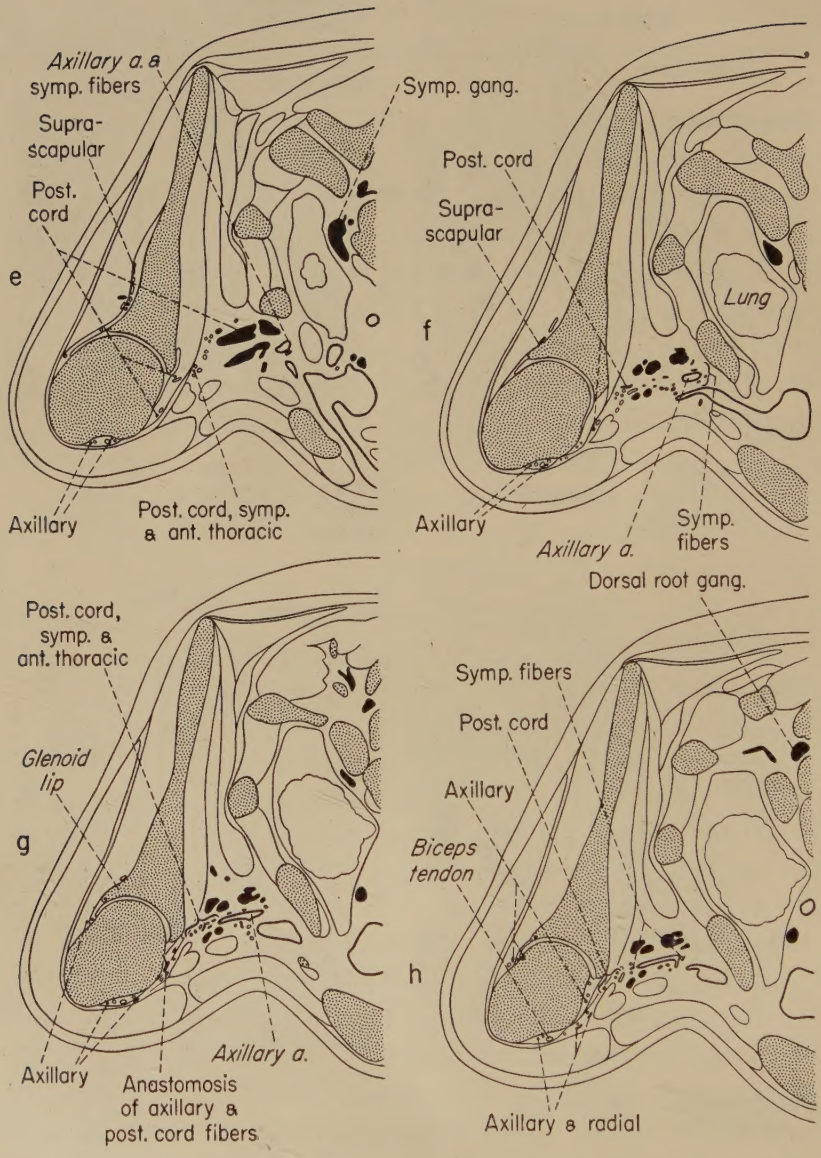

Fig. 2, e-h Continuation of the fetal sections.

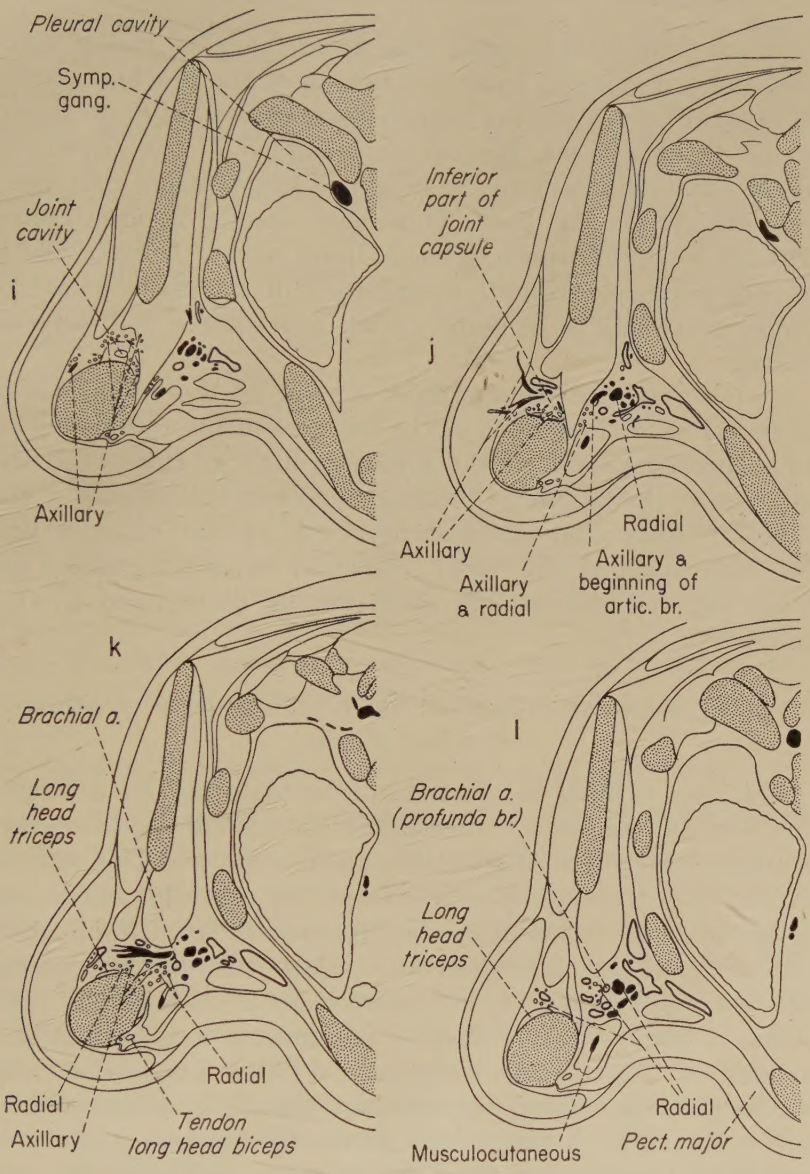

Fig. 2, i-l Continuation of the fetal sections.

The possible articular branches of the radial nerve

In one fetal joint a small twig arose from the radial nerve near the origin of the profunda branch of the brachial artery. In another joint a similar branch arose somewhat more superiorly. In both cases, the nerves ascended in the adventitia of the brachial artery to the origin of the anterior circumflex humeral artery (fig. 2j-l). Here each seemed to anastomose with the bicipital branch of the axillary nerve. It was difficult to determine the ultimate distribution of the fibers from the radial nerve and to be certain that they entered the bicipital sulcus.

In the same two fetal joints, a twig of the radial nerve ascended in the long head of the triceps brachii, together with an ascending branch of the profunda artery. It reached the junction of the tendon of the long head and the capsule of the shoulder joint and disappeared just as the capsule was definitely reached (fig. 2k-l). Whether or not the fibers actually entered the capsule could not be determined in this material.

The articular branch of the posterior cord of the brachial plexus

This was initially discovered in fetal material. Its articular distribution had been overlooked in the first dissections since it appeared to supply the subscapularis muscle and its tendon. In the fetal material it arises from the posterior cord, just proximal to its termination and apparently from the portion giving rise to the axillary nerve. It descends obliquely across the ventral surface of the subscapularis muscle (fig. 2f). When it reaches the tendon of this muscle it divides into a number of twigs. One or two of the twigs anastomose with the bicipital filament of the articular branch from the axillary nerve (fig. 2g). A few others pierce the tendon of the subscapularis muscle and enter the anterior region of the capsule. This area is also reached by fibers ascending from the articular branch of the axillary nerve. Fibers from both

nerves ramify mainly in the fibrous layer of the capsule, but some of them penetrate deeply to the synovial layer in company with blood vessels. In the region of division of the articular branch of the posterior cord, a small group of fibers detaches itself and anastomoses with a group of fibers whose origin is discussed below. The combined bundle of fibers ascends in company with blood vessels to the joint capsule superior to the tendon of the subscapularis muscle and posterior to the coracoid process (fig. 2c-h). Here it ramifies in an area characterized by a rather extensive vascular network. The same area is also reached by fibers from the suprascapular and anterior thoracic nerves.

The articular distribution of this branch of the posterior cord was later confirmed in dissections. It corresponds in most respects to the upper branch of the axillary nerve described by Rüdinger (1857). It is not unlikely that a larger series would show it arising from the axillary nerve as well as from the posterior cord. Only figure 1d, ant. shows the articular branch from the posterior cord in its gross relationships. The other dissections illustrated were done before the existence of this branch was realized.

The articular branches of the anterior thoracic nerves

An articular branch of the lateral anterior thoracic nerve was first noted in fetal joints and subsequently confirmed in dissections. In its gross distribution it accompanies a branch of the thoracoacromial artery, crosses the coracoid process superiorly and reaches the acromioclavicular joint (fig. 1d, ant.). During the terminal part of its course it gives a twig to the anterosuperior region of the joint capsule. In one instance, a very slender filament arose from the main articular branch soon after its origin and ran laterally, anterior to the coracoid process, and entered the bicipital sulcus (fig. 1d, ant.). The other dissections illustrated in figure 1 were done before the existence of the articular branch of the lateral anterior thoracic nerve was realized.

The distribution described above is similar to that found in fetal joints in which it was observed that the fibers enter the anterosuperior region of the capsule. Here they form a number of small bundles, some of which ramify in the fibrous layer where they overlap the area of distribution of the upper articular branch of the suprascapular nerve. The ramification is similar in arrangement to that formed by the axillary nerve and posterior cord in the anteroinferior region of the capsule, although the numbers of nerve bundles are not as great. Other fibers from the articular nerve penetrate deeper into the capsule and become closely associated with the vascular network in the synovial tissue posterior to the coracoid process. This region is also reached by fibers whose origin is as follows.

In one of the fetal joints an additional articular twig of the lateral anterior thoracic nerve was found. This twig, which was not found in any of the dissections, accompanied small blood vessels across the subscapularis muscle and anastomosed with sympathetic fibers descending in the adventitia of the axillary artery. The combined trunk anastomosed with a twig from the articular branch of the posterior cord and the bundle of fibers resulting from this union ascended to the vascular area posterior to the coracoid process (fig. 2c-h). The contribution of the posterior cord was present in all the fetal joints, while that of sympathetic fibers was found in two fetal joints.

Sympathetic fibers

In two of the fetuses sympathetic fibers were traced into the shoulder joint. In one, they arose from the stellate ganglion; in the other from the sympathetic trunk just superior to the stellate ganglion. In both, the fibers descended in the adventitia of the subclavian and axillary arteries to the origin of vessels ascending to the vascular area of the joint. Here they entered into the formation of the ascending nerve bundle described above. In one joint the sympathetic fibers anastomosed with a twig from the lateral anterior thoracic nerve.

Thus there is a direct path for sympathetic fibers to the shoulder joint. Whether this is a fairly constant feature cannot be stated at this time. In the other two fetal joints, fibers of similar origin were found, but they were not as well stained and were lost in the adventitia of the axillary artery.

The articular branches of the suprascapular nerve

As this nerve enters the supraspinous fossa it gives a branch which runs laterally, deep to the tendon of the supraspinatus muscle. During its course it gives a twig to the periosteum of the coracoid process and frequently one to the coracoacromial ligament (fig. 1, post.). It ends as fine rami in the superior region of the capsule, near the cartilaginous glenoid lip. In the fetal joints, this nerve accompanies branches of the transverse scapular artery into the fibrous layer of the capsule near its fusion with the tendon of the supraspinatus muscle (fig. 2a-b). It ramifies in this area and reaches anteriorly into the distribution of the articular branch of the anterior thoracic nerve, and probably into the vascular area as well. The number of nerve fibers in the superior region of the capsule is exceeded only by those in the anteroinferior area. The articular branch also gives a twig to the acromioclavicular joint. The fibers from the suprascapular nerve to the supraspinatus muscle reach as far as its tendinous insertion but it was not possible to determine whether any of them continued into the capsule. Sympathetic fibers were observed accompanying the transverse scapular artery but it was not possible to trace them to the point of origin of articular vessels.

Just before entering the infraspinous fossa, the suprascapular nerve gives a twig to the neighboring periosteum and one or more filaments which run laterally and enter the capsule deep to the tendons of the infraspinatus and teres minor muscles (fig. 1, post.). In the fetal joints, these fibers, together with the blood vessels which they accompany, ramify in the capsule adjacent to the glenoid lip (fig. 2c-f). Small bundles

of fibers occasionally enter the synovial tissue. The lowermost bundles overlap the area reached by fibers ascending from the articular branch of the axillary nerve.

DISCUSSION

There are certain features of the nerve supply of the shoulder joint which in many respects are similar to those of the knee and hip. The nerve supply is characterized by variation in gross distribution of nerves, by regional differences in intra-articular distribution and by a certain amount of overlap of articular nerves.

Variation in distribution of articular nerves is evident mainly in the manner in which they reach the joint. Once within the joint there is a basic pattern of distribution in that each nerve usually supplies a specific region of the joint. The absence of certain articular branches, as determined by dissection, is more apparent than real since many of these are small and easily overlooked in dissection. For example, the bicipital branch of the axillary nerve was found in but one of the dissections, yet was present in all of the fetal joints. The bicipital branch of the lateral anterior thoracic nerve is less constant. According to Arkhangelsky ('31) it is present in 40% of the cases. In this series it was found in but one of the dissections. Also according to Arkhangelsky, at least one branch from the lateral anterior thoracic nerve supplies the superior region of the shoulder joint. In the present study it was found in both gross and microscopic material, so there is little doubt that it is usually present. The other articular branch of the lateral anterior thoracic nerve, that anastomosing with sympathetic fibers and fibers from the posterior cord, was present in but one of the fetal joints and is undoubtedly quite inconstant.

Rüdinger (1857) described an upper branch from the axillary nerve to the anterior region of the capsule. In the present study the branch which corresponds in distribution to Rüdinger's description arose from the posterior cord, although from that portion of the cord giving rise to the axillary

nerve. Arkhangelsky did not mention such a branch, either from the axillary nerve or from the posterior cord. In gross material, this branch appears to supply the subscapularis muscle and its tendon. The fetal material, however, showed that it either pierces the tendon or courses below it to reach the capsule. It may be, therefore, that he assumed it to be a muscular branch. It is quite likely that a series larger than the present one would show it arising either from the posterior cord or the axillary nerve.

Each nerve entering the shoulder joint supplies a definite region, but there is a certain amount of overlap with other nerves, so that each region is supplied by more than one nerve. Thus, the anteroinferior region of the joint is supplied by the axillary nerve, by the posterior cord and possibly by the radial nerve. The anteroposterior region is supplied by the posterior cord, the lateral anterior thoracic nerve and the suprascapular nerve.

Many of the articular nerve fibers undoubtedly supply blood vessels in the capsule and neighboring bone, since they form bundles which are closely associated with the vessels and decrease in size as the vessels decrease. A number of the fibers are undoubtedly vasomotor. This is borne out by the presence of a direct path from sympathetic ganglia to articular vessels. A similar direct path probably exists for the hip joint (Gardner, '48). Fibers from lumbar ganglia reach the femoral artery but their ultimate distribution is uncertain since the artery receives fibers directly from the femoral nerve. The resulting mixture of fibers does, however, contribute to articular vessels. In none of the fetuses could sympathetic fibers descending in vascular adventitia be traced more distally than the hip or shoulder.

A number of bundles of nerve fibers ramified mainly within the fibrous layer of the capsule. This arrangement was particularly striking in the anteroinferior and anterosuperior regions of the capsule. These regions would be most subject to compression or change of some sort during movement. Thus, a situation similar to that in the knee and hip joints

is present. In these joints, the fibrous layer of the capsule in the posterior region of the knee joint and the anteromedial region of the hip joint contains many small bundles of nerve fibers. In the knee joint of the cat, the posterior region of the capsule contains Ruffini-type nerve endings (Gardner, '44).

The functional implications of articular nerve supply were discussed in more detail in the study of the nerve supply of the knee joint (Gardner, '48).

SUMMARY

The nerve supply of the human shoulder joint was studied in dissections and in serial sections of fetal joints. The articular nerves arise from the axillary, suprascapular and lateral anterior thoracic nerves, from the posterior cord, from sympathetic ganglia and possibly from the radial nerve.

The articular branch of the axillary nerve enters the inferior region of the capsule where it ramifies and from which fibers extend laterally into the bicipital sulcus and superiorly in the anterior and posterior regions of the capsule. Recurrent branches from the radial nerve possibly reach both the bicipital sulcus and the inferior region of the capsule.

The articular branch from the posterior cord supplies mainly the anterior region of the capsule, extending both superiorly and inferiorly. It anastomoses with the bicipital branch of the axillary nerve, and also with sympathetic fibers and occasionally a twig from the lateral anterior thoracic nerve. The bundle of fibers resulting from union with the latter two groups ascends to the anterosuperior region of the capsule, especially to the vascular synovial tissue posterior to the coracoid process.

A branch of the lateral anterior thoracic nerve supplies the acromioclavicular joint and during its course gives a twig to the anterosuperior region of the capsule. In one dissection, this nerve sent a branch to the bicipital sulcus.

In two of the fetal joints, fibers from the stellate ganglion and sympathetic trunk coursed in the adventitia of the sub-

clavian and axillary arteries, anastomosed with the branch from the posterior cord, and eventually reached the vascular area posterior to the coracoid process.

The suprascapular nerve gives an upper branch to the periosteum of the coracoid process, the coracoacromial ligament and the superior region of the capsule. A lower branch of the suprascapular nerve supplies the posteroinferior region of the capsule.

Most of the articular fibers probably supply blood vessels in the capsule and neighboring bone. The fibrous layer of the anteroinferior and anterosuperior regions of the capsule contain numerous small bundles of nerve fibers.

ACKNOWLEDGMENTS

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INDIVIDUAL CELLS UNDER PHASE MICROSCOPY BEFORE AND AFTER FIXATION[1]

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TWENTY-ONE FIGURES

While most of the methods of fixing and staining cells are designed to make certain structures more easily visible through the microscope, many questions remain with regard to which structures are artifacts produced by the histological methods, and which methods yield preparations most representative of the living cell.

Several investigators, using tissue cultures or smears of living tissues, have watched and described the process of fixing and staining from the living condition to mounting, using ordinary bright-field microscopy (Lewis and Lewis, '24; and Belar, '30). But with the development of phase microscopy, which allows us to observe very distinctly certain structures in the living cell heretofore often invisible or barely discernible without special histological procedures, a more complete evaluation of the various methods of fixation, staining, and other stages in the preparation of cells for microscopic study is made possible.

This paper describes the use of phase microscopy for determining which methods of fixing least alter the appearance of living cells in tissue culture.

[1] The author is indebted to Dr. William Bloom, Department of Anatomy, for the use of his laboratory during the course of this work. This study was aided by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of The University of Chicago.

MATERIALS AND METHODS

The salamander macrophage was selected for this study because it is a very large cell, and it can be maintained easily in tissue cultures at room temperatures. In cultures, numerous macrophages flatten out very thinly against the coverslip within a few days after explanation. To make the cultures, fragments of spleen, liver, and testis of salamanders (Triturus) were explanted aseptically into Tyrode's solution diluted with distilled water to isotonicity with amphibian blood. Other cultures were made in diluted White's liquid medium (White, '46). Cells continued in good condition considerably longer in White's medium than in Tyrode's solution.

To prepare a culture for the study of a particular fixative, the explant was removed to avoid the scattering of light which occurs in thick preparations under phase illumination. Then the coverslip was placed with the residual migration zone of the culture face down on a 2 × 3 inch glass slide. The cells were protected from crushing by a support consisting of two strips of no. 1 coverslip, one at each side and arranged parallel to the long axis of the slide. After a cell was observed and photographed while still alive and moving, a fixing solution was run under the coverslip and the cell photographed again. Bits of filter paper placed at the edge of the coverslip opposite the point where the solutions were introduced helped draw the solutions through rapidly. After fixation, the preparation was washed by introducing water, or alcohols depending on the fixative used, at the edge of the coverslip in the same manner as was used for the fixing solution. In this way the same cell was observed and photographed (using a Leica camera with Ibso attachment; 8 × ocular; 95 × 1.25 N.A. bright-field objective and 95 × N.A. 1.8 (0.16 A–0.25 λ phase objective) during each of the remaining histological processes: staining, dehydrating with 50, 70, 95, and 100% alcohol, then clearing in xylol, and mounting in clarite.

A number of fixing agents was studied: absolute alcohol, Carnoy's solution, Bouin's solution, 10% acid formol, 10% alkaline formol, Helly-Maximow solution (9 parts Zenker

stock solution to one part formol, pH 8 — Zenker-formol) and Maximow's fluid (9 parts Zenker stock solution, one part formol, pH 8, and one part 2% osmic acid — Zenker-formol-osmic). Delafield's hematoxylin was the principal stain used. In a few instances Sudan black B and Nile blue sulfate were tried.

OBSERVATIONS

Under *bright-field illumination* the salamander macrophage usually appeared as a disc-like cell with a spherical or flattened ovoid nucleus (fig. 2). The cell membrane was frequently not visible in its entire periphery. It was particularly difficult to see where it appeared to have made contact with the glass surface and had become extremely thin or drawn out into very fine processes. Indistinct wavy lines appeared on the surface of the cell; these are seen more clearly under phase and are described in the paragraph below. Among the cytoplasmic inclusions, the fat droplets were the most prominent and clearly defined. Mitochondria could be seen as hyaline dots, rods or filaments, grayish with apochromatic objectives, and more or less crowded. They were usually grouped in a radiating fashion about what was probably the cell center. Centrioles were not visible. If pigment granules were present they showed up clearly. The remaining cytoplasm was clear or had a faintly granular appearance. The nucleus had a clearly visible nuclear membrane and contained some barely discernible irregular and vaguely outlined patches of chromatin material. One or more well-defined nucleoli were usually seen in the macrophage nucleus.

Under *phase microscopy* the living salamander macrophage presented a rather different picture from that under ordinary bright-field microscopy. With the dark-contrast objective specified above, the cell boundaries appeared distinct all around the periphery (fig. 1). Even very tiny filose pseudopods and very thin membranes were clearly differentiated from the lighter gray background. The indistinct wavy lines seen under the bright-field microscope now appeared as definite folds, sometimes as pseudopods between the cell and

coverslip. The cytoplasm contained numerous granular inclusions, although in many cells in thin areas the ground substance was clear and structureless. Fat droplets, so clearly demarcated under bright-field, looked like brilliant little suns, some with dark centers, and they obscured immediately adjacent structures in a manner especially disadvantageous in photomicrography. Mitochondria showed up as tiny black dots, short rods, granular chains, or straight or curved black filaments. The cell center, which was not clearly visible as such under bright-field illumination, was a clear light gray area frequently surrounded by radiating lines of mitochondria. In some cells it was possible to see the centriole as a small grayish body, sometimes as a diplosome. The living nucleus under phase looked almost like a fixed and stained nucleus (fig. 3). The nuclear membrane was sharp and well defined. The nucleolus appeared dark and the nucleus was filled with irregular sharply outlined patches of dark material which was shown after fixation and staining to be chromatin. See figures 1, 2, and 3.

Cells were observed while fixation, washing, staining, etc., was carried out (plate 2). The structural changes were observed with much greater clarity under phase than under bright-field microscopy. In general, cytoplasmic structures, except mitochondria, appeared distinct, particles had sharper boundaries and there were greater contrasts between particles and the background. Fine particles and edges of membranes were black against a light gray background. During fixation the cell boundary became a little more prominent, and, like all of the other structures it remained very much as it was in the living or it changed very considerably depending upon the fixing solution. The action of particular fixing solutions will be noted later on. During the penetration of the fixing agent into the cell there was considerable agitation of particles. The ground plasm became finely granular. This activity lasted only a few seconds, after which shrinkage occurred with some fixatives. The cellular inclusions such as the mitochondria were sometimes well preserved or were con-

siderably changed or even destroyed as a result of the action of certain fixatives. The nuclear structure became more definite with great suddenness. Chromatin appeared as clearly defined irregular clumps joined together by irregular, usually thin strands. With certain fixatives there was little alteration of the main structure as seen under phase microscopy (figs. 4 and 5). But under the bright-field microscope the nucleus suddenly became full of chromatin clumps, resembling the usual picture of a fixed and stained preparation. Washing the cell free of fixative changed the cell appearance practically not at all (fig. 6).

Staining with diluted aqueous Delafield's hematoxylin gave the typical picture of a stained cell (fig. 7). Again, staining seemed to reveal nothing more to the eye observing under phase; staining made a great deal of difference while observing the process under the bright-field microscope. Gradual dehydration through a series of alcohols to absolute alcohol removed fat droplets, leaving empty vacuoles. Otherwise, dehydration produced little change except for a slight degree of additional shrinkage. Compare figures 5 and 8. Clearing in xylol and mounting in clarite produced additional shrinkage and tended to render some of the cytoplasmic structures less easily visible under phase then they were in alcohol or water (fig. 9). After the cell was mounted in clarite no more detail could be seen under the particular phase used here than under the usual bright-field microscope.

Since the fixing agent played by far the greatest rôle in the final appearance of the preparation and was responsible for the greatest part of the shrinkage, the effect of each fixing agent tried will be described. In each case the same sort of cell preparation was used; hence, the results are all fairly comparable.

Absolute alcohol introduced under the coverslip produced an especially violent reaction in the cytoplasm, reminding one of the appearance of an egg being fried in an overheated pan. The cytoplasmic structures were seriously distorted. Mitochondria and fat droplets were destroyed. The nucleus

shrank approximately ⅐ of its diameter — a greater degree than was noted for any other of the fixing agents tried. See figures 10 and 11. *Carnoy's solution* (figs. 12 and 13) and *Bouin's solution* (figs. 14 and 15) gave about the same sort of picture as did absolute alcohol, although Carnoy's solution produced slightly less shrinkage of the nucleus than absolute alcohol and Bouin's considerably less shrinkage of the nucleus than absolute alcohol. After Carnoy's and Bouin's solutions, although the mitochondria were destroyed, the cell stained brilliantly and looked clean and sharp when mounted in clarite; the final product, however, was a greatly altered cell compared with its appearance in the living under phase.

Ten per cent formol, pH 3.7, preserved the cell better than absolute alcohol, Carnoy's solution or Bouin's solution. There seemed to be less overall disturbance to the cell outlines and contents. Chromatin material appeared to be retained fairly well (figs. 16 and 17). *Ten per cent formol, pH 8,* saturated with magnesium carbonate, produced an apparently well-fixed preparation which retained the cytoplasmic structures and texture more closely than any of the above. However, during fixation the cell gave off droplets of material (clasmatosis) which remained fixed in the vicinity of the cell (figs. 18 and 19). Acid formol produced a somewhat greater shrinkage of the nucleus than did alkaline formol.

The best fixing agents of the group here tried were *Zenker-formol* and *Zenker-formol-osmic.* These produced very little change in the appearance of the cell (figs. 20 and 21). Mitochondria, centrioles, and granules seen in the living cell were well preserved. The nucleus showed the least shrinkage and internal change after these fixatives than after any of the others. Granule for granule those in the living nucleus could be identified with those in the fixed preparations and this was often true for the cytoplasmic structures. When Zenker-formol-osmic solution was used, the results of fixation resembled the appearance of the living cell more closely than when Zenker-formol solution was used, and, in addition, the fat droplets were retained because of the osmic acid content

of the fixing solution. While the nucleus did not stain as brilliantly nor as deeply with Delafield's hematoxylin after fixation with Zenker-formol-osmic solution, the nucleus seemed to be more faithfully preserved than when the osmic acid was omitted. See figures 4, 5, 6, 7, 8, 9, 20, 21.

DISCUSSION

The phase-microscope picture of the structure of the untreated living cell confirms in large part the conception of cell morphology as determined on the basis of what have been generally thought were the better cytological methods. Among the group of fixing agents tried, absolute alcohol, or those containing acetic acid (Carnoy's and Bouin's solutions) produced the most serious distortion of the cytoplasm although less of the nucleus. Formol alone, particularly alkaline formol, was better. However, those fixing agents containing potassium dichromate, mercuric chloride, formol, and especially osmic acid, yielded fixed images most like the living cell (see fig. 2, Maximow and Bloom, '42).

From these observations it is clear that the vague chromatin masses seen in the nucleus under bright-field microscopy, and much more precisely seen under phase, actually are well defined chromatin particles which exist in the living cell much as they are demonstrated in suitably fixed and stained preparations.

Evidence is presented that the centriole can be seen and photographed in the living macrophage of Triturus by phase microscopy as a grayish body, sometimes clearly double, which becomes considerably darker and stands out boldly in the fixed cell (even without staining).

Mitochondria are seen under phase in the living cell as prominent easily visible black dots, rods, or filaments against a light gray background. These structures remain in properly fixed cells.

While phase microscopy has not revealed any structures beyond those known in these cells on the basis of the better methods of fixing and staining, it has increased the validity

of our knowledge of cell structure derived from fixed and stained materials. These observations show that certain fixatives present the fixed cell in close structural agreement with the observations made on the same living cell under phase microscopy and that other fixatives produce great distortion.

The greater detail obtained in phase photomicrographs makes possible detailed photographic records over a period of time of the same living cell without resorting to fixing and staining.

SUMMARY

1. Cytological details were seen more clearly in the living salamander macrophage under phase than under ordinary bright-field microscopy; such were mitochondria, cell membranes, and nuclear details. The centrioles, not seen at all under bright-field, became apparent under phase. Fat droplets were more difficult to make out clearly under phase, and tended to obscure immediately adjacent details because they scattered light.

2. Individual macrophages of the salamander were observed in vitro and photographed under phase, first alive and subsequently during fixation, staining, dehydration, clearing, and mounting.

3. The validity of several histological procedures was checked by using phase microscopy to give a more detailed picture of the living cell as the basis of comparison with the cell picture produced by fixing agents.

4. Of the fixing solutions used, Zenker-formol-osmic and Zenker-formol solution yielded preparations resembling living cells much more closely than did formol (either acid or alkaline). In turn, alkaline formol was superior to Bouin's solution, Carnoy's solution and absolute alcohol.

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PLATES

EXPLANATION OF PLATES

All photomicrographs except figures 2 and 13 were made employing a Spencer phase microscope oil immersion objective 95 ×, N.A. 1.8 (0.16 A–0.25 λ), 8 × ocular. Figures 2 and 13 were made using an oil immersion objective 95 ×, N.A. 1.25 and 8 × ocular.

PLATE 1

EXPLANATION OF FIGURES

1 Living macrophage in samander (Triturus) spleen tissue culture under phase. × 1100.

2 Living macrophage under bright-field microscopy. × 1100.

3 Same cell as in figure 2, a few moments later under phase. Compare, in figures 2 and 3, nuclear details, mitochondria, fat droplets. × 1100.

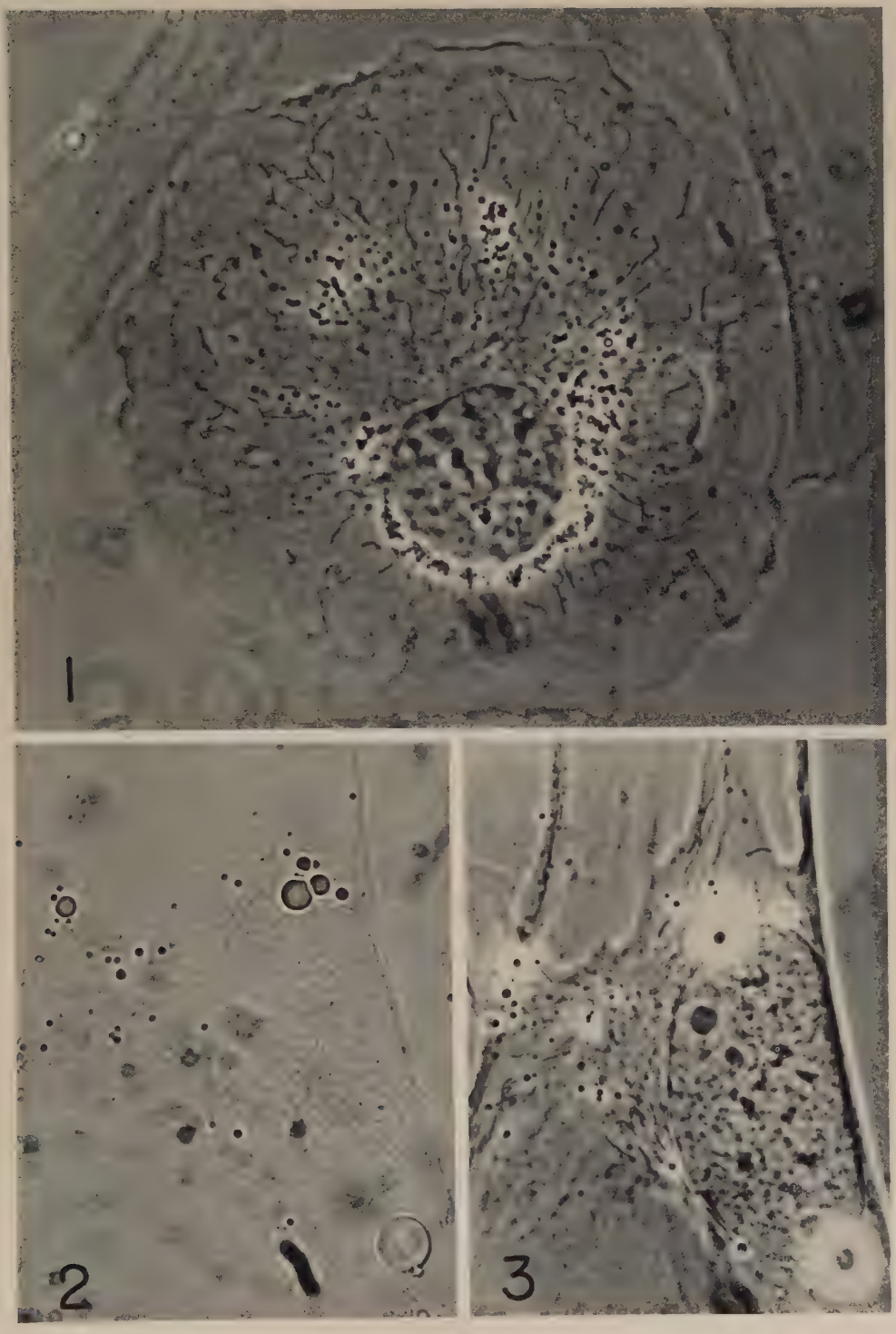
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PLATE 2

EXPLANATION OF FIGURES

Macrophage in salamander spleen tissue culture; all figures of the same cell. Phase × 800.

4 Living cell.

5 Fixed with Zenker-formol solution. Note prominent diplosome near center of cell.

6 Washed with water.

7 Stained with Delafield's hematoxylin.

8 Dehydrated with absolute alcohol (after running up through 50, 70, and 95% alcohol).

9 Cleared in xylol.

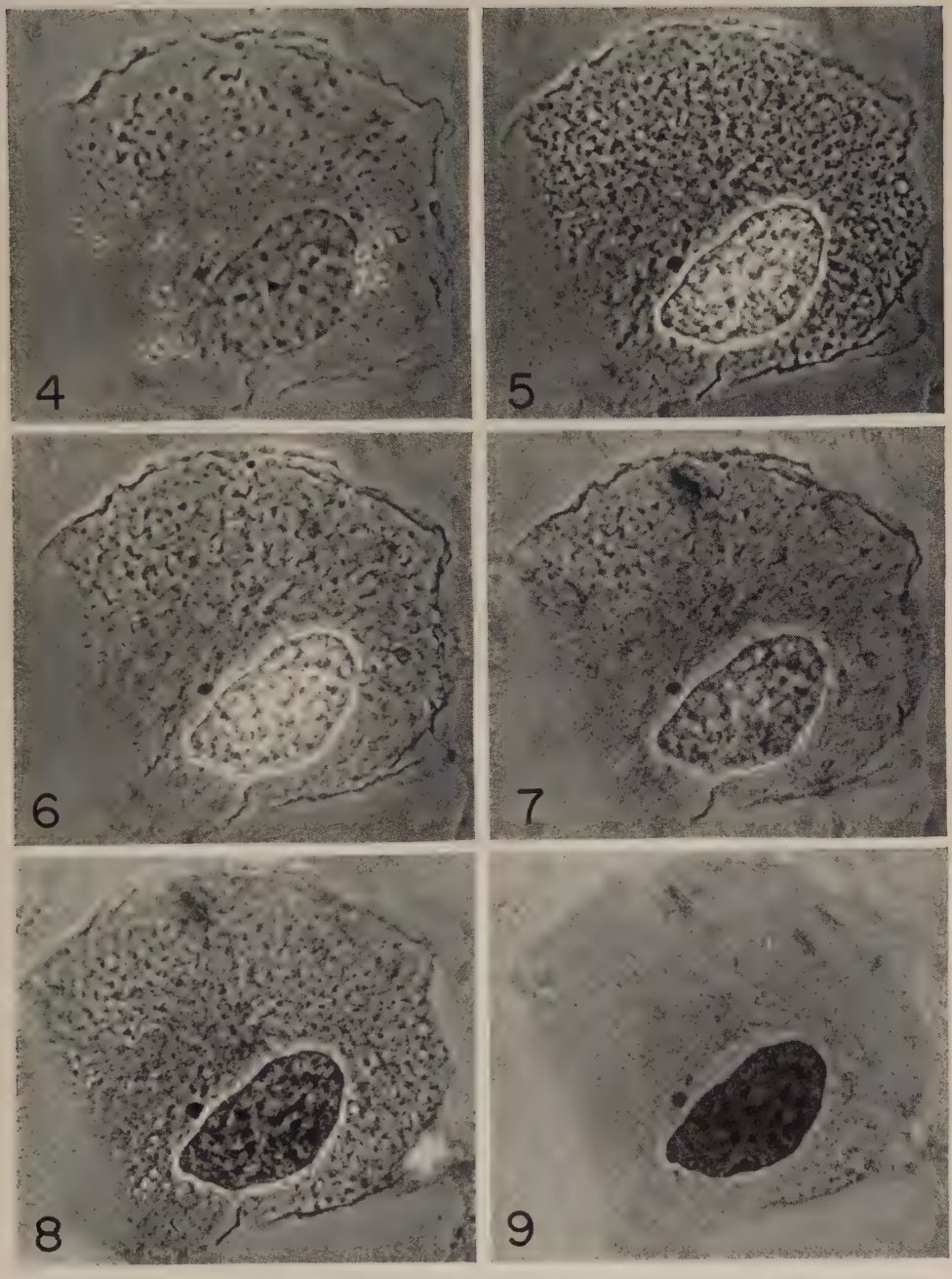
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PLATE 3

EXPLANATION OF FIGURES

10 Living macrophage from salamander spleen tissue culture. Phase, × 670.

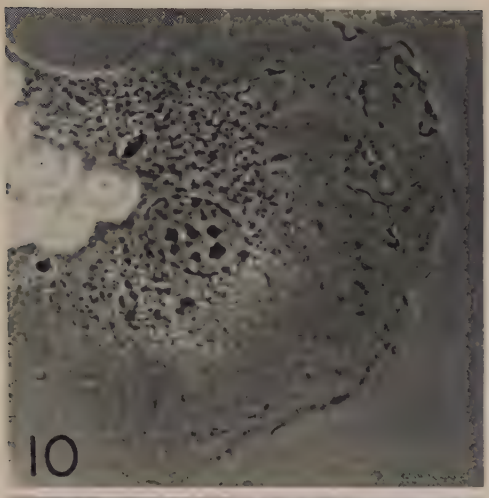
11 Same cell, fixed in absolute alcohol. Note granular appearance of cytoplasm and nuclear changes. Phase, × 670.

12 Living macrophages from salamander liver culture. Phase, × 980.

13 Same cells, fixed in Carnoy's solution, stained in Delafield's hematoxylin, and mounted in clarite. Photomicrograph made through bright-field microscope. × 980.

14 Living macrophage from salamander spleen culture. Phase, × 670.

15 Same cell, in absolute alcohol after fixation in Bouin's solution and washing in 90% alcohol. Note the granular cytoplasm. Phase, × 670.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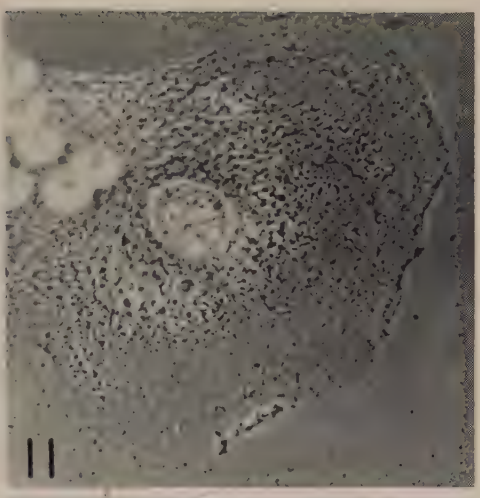
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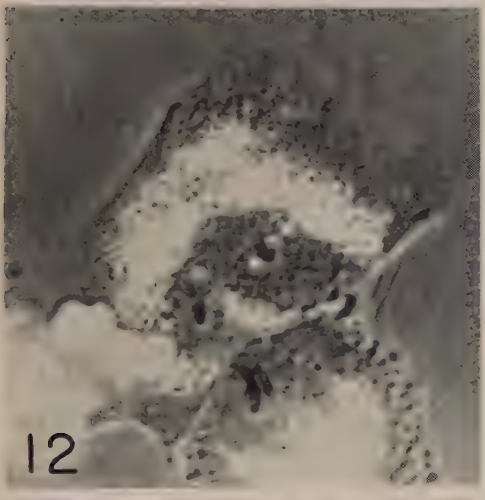
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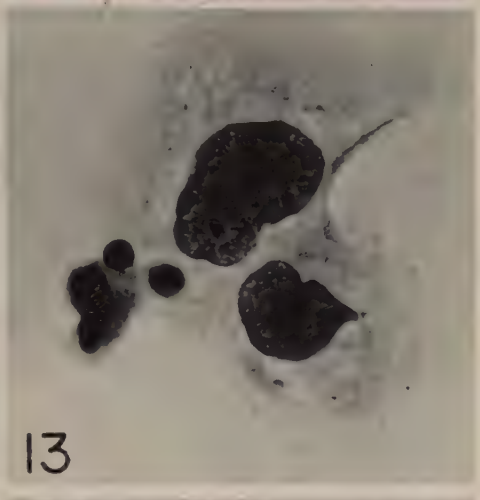
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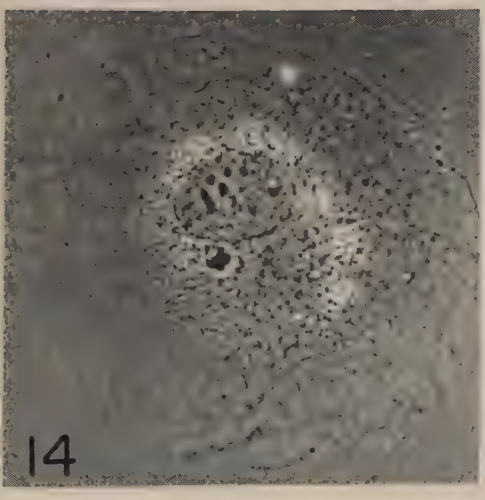
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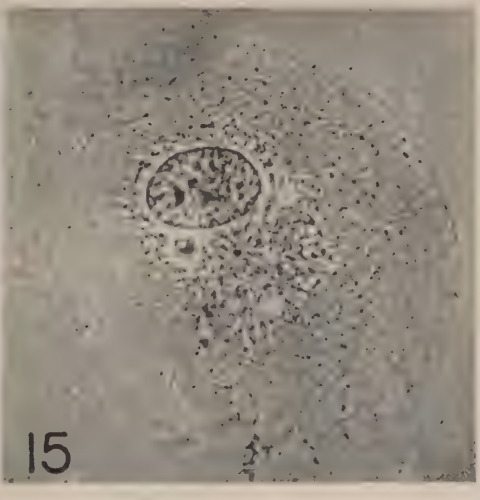
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PLATE 4

EXPLANATION OF FIGURES

16 Living macrophage in salamander testis culture. Phase. × 670.

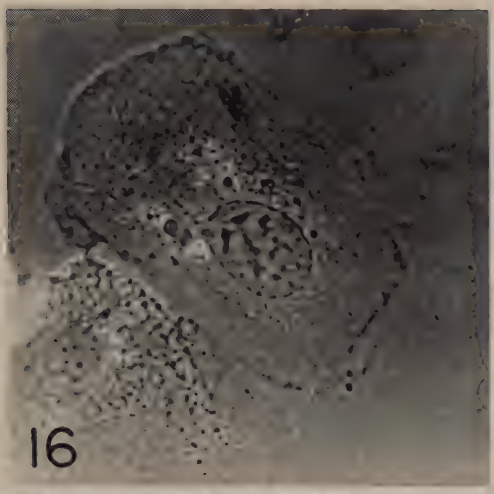
17 Same cell, in 10% formol, pH 3.7. Phase, × 670.

18 Living macrophages in salamander testis culture. Phase, × 670.

19 Same cell, beginning to fix in 10% formol, pH 8.1. Note clasmatosis. Phase, × 670.

20 Living macrophage in salamander spleen culture. Phase, × 670.

21 Same cell, cleared in xylol after fixation in Zenker-formol-osmic solution, washing, staining, and dehydrating. Note the close correspondence with figure 20 Phase, × 670.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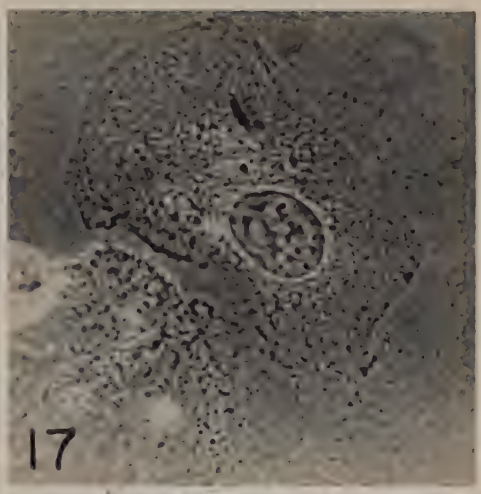
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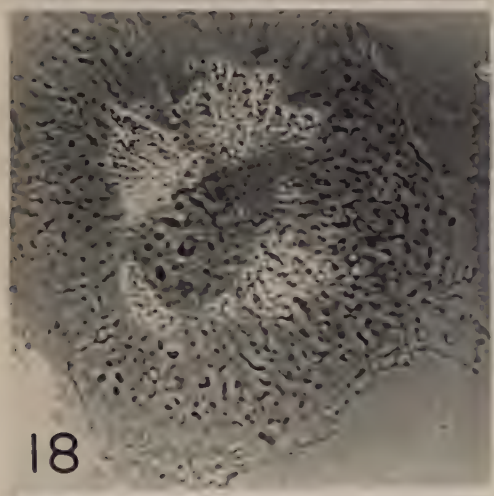
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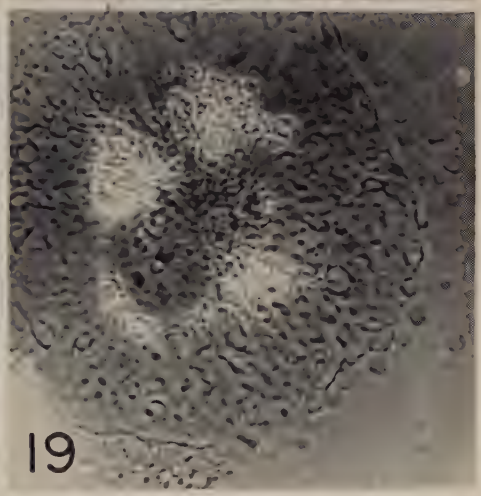
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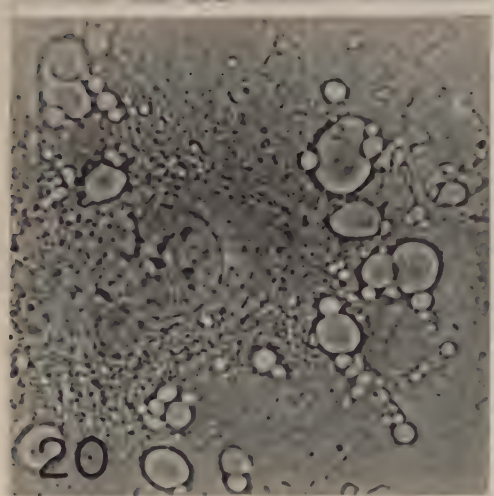
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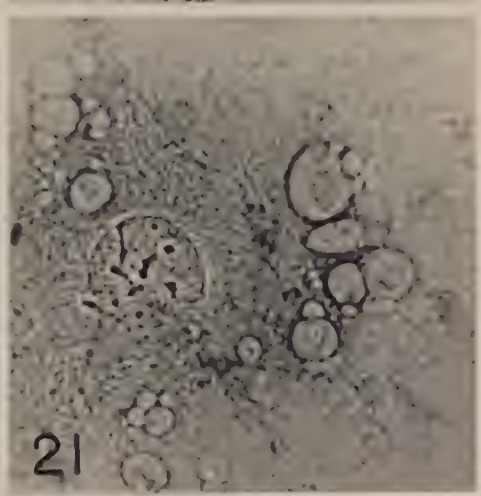
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NILE BLUE STAINING OF ADRENAL GLANDS OF LIVING MICE[1]

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FIVE FIGURES

The blue staining that occurs in malignant cells (sarcoma, carcinoma and lymphoma) and in the thymus, lymph nodes and testicles of mice receiving diets containing Nile blue dyestuff is characterized by diffuse coloration of the entire cell rather than by staining of concrete granules within the cells as occurs in vital and supravital staining (Lewis, Sloviter and Goland, '46; Lewis, Goland and Sloviter, '46; and Lewis and Goland, '47). There are, however, a few exceptions to this for certain granules in the adrenal glands, the intestinal mucosa, the kidney tubules, the prostrate gland and in some macropages, do become stained blue, although the granules that become colored following administration of Nile blue are not the same as those that stain with vital dyes such as janus green, neutral red, methylene blue and trypan blue. For example granules in cells of the islands of Langerhans become deeply colored with neutral red, but not with Nile blue.

Two types of Nile blue granules appear in the adrenal glands. One (fig. 1) corresponds to the pigment granules of brown degeneration first described in adrenals of mice by Cramer and Horning ('37), and later by Tobin and Brinbaum ('47). The other (fig. 2) resembles those described as ascorbic acid granules in chick embryos by Barnett and Bourne ('41), and in the adrenals of rats by Dean and Morse ('48).

[1] Aided by a grant from the National Cancer Institute, Bethesda, Maryland.

Fig. 1 Photograph of a hand-cut section of living adrenal gland from an adult mouse that had received 0.2% Nile blue BB for 3 days. Shows blue bodies in cells located at the junction of the cortex and medulla. Cells with blue bodies that appear to be within the medulla were in reality in a layer just above it. The large cells contained large blue bodies while the smaller bodies were in small cells. The cortex was comparatively free of blue granules. Nuclei of living cells are not shown.

In mice receiving a diet containing Nile blue, one of the earliest indications of its affinity for certain cellular substances is the appearance of blue bodies in macrophages located in the adrenal glands along the cortico-medullary junction, and owing to the resistance to putrefaction of the adrenal glands, blue color persists in cells in this region long after the death of the animal. The majority of blue containing cells lie at the corticomedullary junction, but in advanced stages they spread into the cortex and also into the medulla (figs. 2 and 4). The amount of blue in adrenals of mice of different ages corresponds so exactly with the amount of pigment shown by Tobin and Brinbaum[1] that their figures could be used to illustrate this type of Nile blue bodies. The specific granules characteristic of adrenal gland cortex and medulla do not become stained with Nile blue.

In mice fed Nile blue for 24 hours the blue bodies were of various shades, from pale to dark blue, and traces of hemoglobin were present in some of the paler ones showing that they were remnants of ingested red blood cells. In old adult mice there were large masses of granular material stained pale turquoise while smaller granules in the same cells were deep blue. Differences in shades of blue were not so evident in mice fed with Nile blue for several days.

Tobin and Brinbaum state that mice fed high lard diets deficient in vitamin E had little brown degeneration in their adrenal glands when given 10 to 15 mg of tocopherols orally once a week, or when tocopherols were incorporated in their diet to a concentration of 1%. Increasing the oral dose of tocopherols to a content of 3% resulted in complete absence of adrenal pigment. Since the Nile blue stained bodies present in adrenal glands seemed to correspond to brown degeneration, an effort was made to inhibit their appearance by adding tocopherols to diets containing Nile blue dyestuff.

METHOD

Nile blue 2B, Colour Index 914, both Nile blue 2B (Calco Chemical Division, American Cyanamid Co.) and Nile blue BB

(National Aniline Division, Allied Chemical and Dye Corp.) were used in these studies. The tocopherols used were pure ephynal acetate (Hoffman-LaRoche, Inc.) and α-tocopherol concentrate (B-576) and α-tocopheryl phosphate (B-585) (Distillation Products, Inc.). The majority of mice used were young, 5 to 6 weeks old, although some old mice were studied for a comparison of Nile blue substances in young and old mice. Diets were prepared by grinding pulverized Purina fox chow together with 0.2% Nile blue and 0.25% to 1.0% tocopherol in an electrically driven ball mill for 20 to 30 minutes in order to insure an even mixture of the ingredients. The mice were kept in separate cans, with free access of water and given 3 to 5 gm of food each day. After a desired period of time (1 to 3 or more days), the mice were sacrificed, their adrenal glands removed to a drop of physiological salt solution and there cut into 6 or more thin slices. The living tissue was then covered with a cover glass and studied under the microscope.

RESULTS

In our experiments using a standard commercial chow, the addition of tocopherols to food containing Nile blue did not result in fewer cells containing blue bodies at the junction of the cortex and the medulla of the adrenals of the treated mice either in young mice or old mice. The number of cells containing blue bodies and the size and number of stained bodies in these cells seemed to depend on the age and condition of the treated mice. We were unable to test the influence of 3% tocopherol, as Tobin and Brinbaum did, because large amounts (2.5% to 3%) of the tocopherols we used proved to be toxic to our mice.

While the number of macrophages containing blue bodies and the amount of stained material in them, in other words the amount of brown degeneration, seemed to depend largely upon the age and condition of the treated mice, there was another type of blue granule present in the cortex of the adrenal glands that varied markedly according to the diet

the mice received; as for instance, mice receiving bactopeptone, amigen, and certain amino acids together with Nile blue had few blue granules in the cortex while mice fed α-tocopherol phosphate together with the same amount of dyestuff had innumerable tiny blue granules in the cortex and also some in the medulla. These tiny dark blue granules were not located near the nucleus as are granules stained with vital dyes, but were distributed throughout the cytoplasm of the cells. In some preparations they were distributed so much at random that the preparations had the appearance of pepper shaken over it (fig. 2). There were more blue granules in cells toward the medullary boundary than toward the outer surface of the cortex, and in some adrenal glands the tiny blue granules were present in great numbers in a rather broad band along its inner border. In some preparations many of the tiny blue granules were scattered throughout the medullary cells as well as the cortical cells. Owing to their size, their distribution in cells, and their presence in the cortex, the tiny blue granules resembled those described as ascorbic acid granules by other investigators, particularly those shown in figures 5 and 6 (Dean and Morse, '48).

The number of tiny blue granules varied independently of the number of cells containing large blue bodies located at the junction of the cortex and medulla. Under certain conditions the cortex was peppered with small blue granules while only a few macrophages with blue bodies were present at the corticomedullary junction. In other instances, only a few tiny blue granules were found in the cortex while many macrophages loaded with large blue masses were present at the junction of the cortex and the medulla and extended into both the cortex and the medulla.

Szent-Györgyi ('28) found that the hexuronic acid (ascorbic acid) had reducing properties stronger than other intracellular agents and was capable of reducing silver nitrate in the dark. Since then Barnett and Bourne, and Dean and Morse have developed a cytological method for the demonstration of ascorbic acid by means of fixation of tissue in an

acid solution of silver nitrate. These investigators claim that while other compounds can reduce silver nitrate at neutral and alkaline hydrogen-ion concentrations, ascorbic acid is the only substance in protoplasm that is known to reduce acidified silver nitrate.

Because of the resemblance to the tiny Nile blue granules in the cortex to the reduced silver granules located in the same site, studies were undertaken to determine whether the two types of granules represent the same material. In these studies thin slices of tissue were placed in acid silver nitrate fixing solution as rapidly as possible after the animals were sacrificed. Following this, microscopical preparations of the living tissues were made using the adrenal from the other side of the mouse and photographed to show the blue bodies. Preparations were made from tissue of mice fed on the usual 0.2% Nile blue diet and from others that received additional treatment influential in regulating the amount of ascorbic acid present in the adrenal glands, as for instance injection of ascorbic acid or adrenotropin.

Photographs of adrenal glands from the same mouse, one of living adrenal tissue with Nile blue stained bodies (fig. 2) and the other of adrenal tissue after fixation with silver nitrate (fig. 3) showed that the tiny blue granules of the living preparation were represented by black granules of the same size, number, distribution and position in the cortex of the silver nitrate fixed preparation.

The large blue granules and masses (fig. 4) in the cells located at the junction of the cortex and medulla were not blackened by silver nitrate (fig. 5). A comparison of living Nile blue preparations with ones treated with silver nitrate, and with others stained with carbolfuchsin according to the method of Tobin and Brinbaum, showed that the Nile blue bodies in the large macrophages that were not blackened by silver nitrate corresponded to those that were stained with carbolfuchsin.

Examinations of microscopical preparations of living adrenal glands of mice that had been fed 0.2% Nile blue for

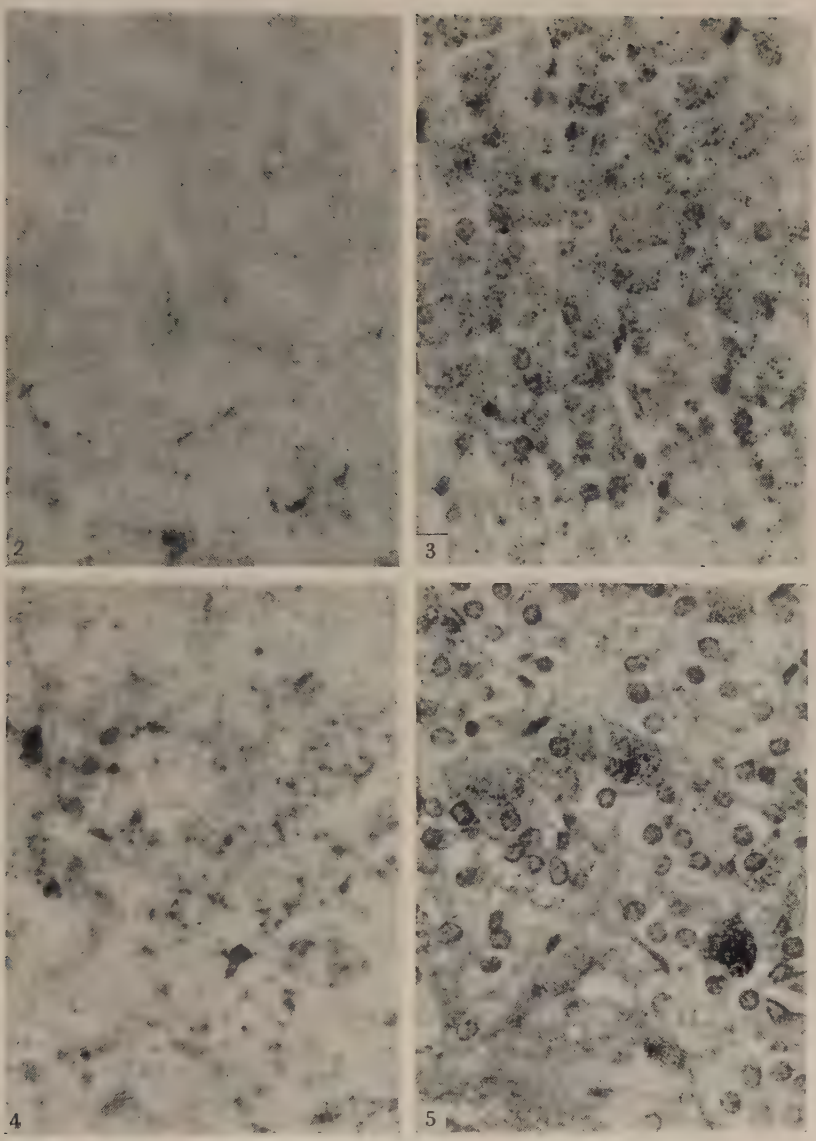

Fig. 2 Photograph of living cortex of left adrenal showing tiny blue granules scattered throughout cells. Nuclei not shown.

Fig. 3 Photograph of section of right adrenal of same mouse fixed with silver nitrate, showing tiny black granules of ascorbic acid.

Fig. 4 Photograph of adrenocortical junction of living left adrenal showing large cells with large and small blue bodies. Nuclei of living cells not shown.

Fig. 5 Photograph of a section of right adrenal gland of same mouse fixed with acidified silver nitrate. Large cells have unstained brown degeneration bodies and some tiny black granules of reduced silver.

one or more days made it possible to estimate the amount of brown degeneration and also the amount of ascorbic acid present in their adrenals.

CONCLUSION

The amount of ascorbic acid and of brown degeneration present in the adrenals of mice can be demonstrated by feeding them 0.2% Nile blue 2B for one or two days. Under these conditions ascorbic acid appears as tiny blue granules scattered throughout the cells of the cortex, sometimes even in the cells of the medulla, while brown degeneration appears as large bodies and granules in macrophages located principally along the corticomedullary junction and in some instances extending into the cortex and also into the medulla.

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SEX DIFFERENCES IN THE DISTRIBUTION OF TISSUE COMPONENTS IN THE HUMAN LEG FROM BIRTH TO MATURITY

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THREE FIGURES

In a previous article (Reynolds, '48) values were given representing the growth of bone, muscle and the subcutaneous tissues (including skin) which overlie the muscle mass in the female leg. These values were obtained from measurements made on roentgenograms taken at 7 age-levels, from birth to maturity.

The present paper presents similar values for males, and describes sex differences in distribution of tissue components in this anatomic area.

The age-levels studied, the partly longitudinal nature of the data, and the techniques used in obtaining and measuring the roentgenograms, all correspond to the earlier study on females. Briefly, measurements are taken from anteroposterior roentgenograms of the calf, at the level of the greatest calf breadth. *Bone breadth* is the sum of shadows of tibia plus fibula. *Muscle breadth* is the sum of breadths of muscle shadows on either side of the bones, plus the interosseous space. *"Fat breadth"* is the sum of breadths of shadows of skin and tela subcutanea. The three breadths when added equal the total x-ray breadth of the calf.

The advantages and limitations of this method of studying tissue differentiation have been discussed, and relevant literature cited, in the earlier study.

The correlation coefficient *(r)* between direct calf circumference and x-ray breadth of calf was calculated at each age-level for the males, as had been previously done for the females, and shows similar results, the values ranging between 0.60 and 0.96.

Means on males for weight, height and calf circumference, measurements taken on unclothed subjects, are shown below. Means for the males in the present study correspond to Fels norms (Sontag and Reynolds, '45) at the earlier age-levels, but the 15.5-year sample is taller and heavier than the group average.

AGE-LEVEL	NO.	WT.	HT.	CALF CIRC.
		kg	*cm*	*cm*
Birth	28	3.36	49.8	10.8
1 month	30	4.27	54.9	12.2
1 year	30	10.40	76.1	19.0
7.5 years	28	26.00	126.0	25.2
12.5 years	29	44.68	152.4	31.6
15.5 years	13	66.11	175.8	36.5
Adult (33.5 years)	31	72.35	177.3	35.5

The mean age of the adult females in the previous study was 23.5 years. Since the mean age of the adult males in the present series is 33.5 years, a new sample of adult females, with an average age of 31.5 years, is used for comparison. Differences between mean values for the two female groups is small:

	FAT	MUSCLE	BONE	TOTAL BREADTH
Previous study	18.9	61.9	32.6	113.4
Present study	19.7	59.7	32.8	112.2

RESULTS

Table 1 gives means and standard deviations for absolute tissue breadths, for both sexes, and a comparison of the means is shown in figure 1.

As seen in figure 1, the sexes show similar patterns for total breadth of calf, and for bone breadth. In muscle breadth,

TABLE 1

Means and standard deviations for absolute tissue breadths

AGE-LEVEL	NO.	FAT		MUSCLE		BONE		TOTAL BREADTH	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
				Males (mm)					
Birth	28	7.4	1.7	18.0	2.2	10.6	1.1	36.0	3.3
1 month	30	9.9	1.6	20.2	2.6	10.8	.7	40.9	3.3
1 year	30	20.2	3.6	30.5	3.5	16.2	1.4	66.9	3.9
7.5 years	28	11.3	2.8	44.1	4.4	27.0	2.0	82.4	5.8
12.5 years	27	14.1	3.4	55.3	5.3	32.8	2.3	102.1	8.8
15.5 years	13	11.0	2.2	68.7	8.7	37.3	3.3	117.1	8.5
Adult	31	9.6	9.0	65.8	6.1	39.5	3.3	114.9	7.8
				Females (mm)					
Birth	30	8.4	1.9	20.2	2.0	7.7	1.1	36.4	3.1
1 month	30	10.5	2.0	19.2	2.0	10.7	.6	40.4	3.3
1 year	30	21.5	2.9	29.9	3.8	15.5	2.1	66.9	4.6
7.5 years	29	13.4	3.4	42.4	4.6	25.2	2.3	81.0	6.4
12.5 years	27	15.7	2.9	52.8	6.8	30.0	2.2	98.5	8.7
15.5 years	13	19.5	4.9	60.3	9.1	32.5	3.3	112.3	14.4
Adult	31	19.7	4.7	59.7	5.3	32.8	1.5	112.2	5.5

the increase at adolescence for males greatly exceeds that for females. Breadth of "fat" (tela subcutanea plus skin) for males shows the same sharp rise in infancy, with a decrease in middle childhood, as was noted previously for females. The sexes show similar patterns in fat breadth until adolescence, when they diverge, the males decreasing and the females increasing.

Sex differences in the distribution of the tissue components are apparent. Males are consistently larger in mean breadths of muscle and bone, and in total breadth of calf. Females are larger than males in mean fat breadth. These differences may

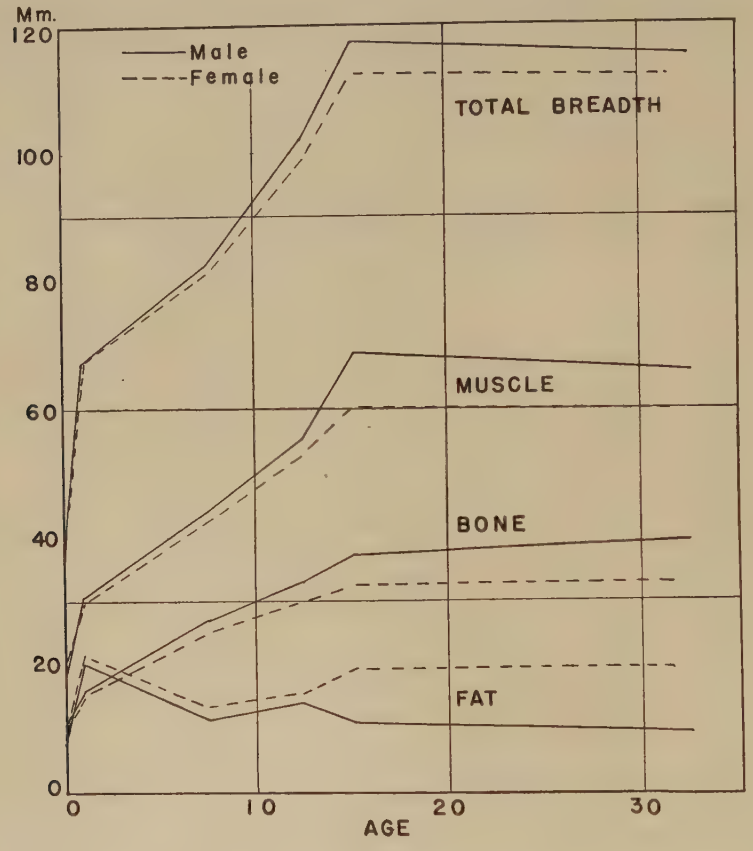

Fig. 1 Means for x-ray breadth of calf and tissues, male and female.

be seen more sharply in the mean relative breadths shown in table 2 and figure 2.

Relative breadth represents the percentage of total breadth of calf occupied by each tissue component. Males have relatively more muscle and bone, and relatively less fat, than females. These differences are pronounced in adolescence and

maturity. For instance, at 7.5 years the percentages of fat, muscle and bone, respectively, are 14, 53 and 33 for males and 17, 52 and 31 for females. By 15.5 years, these values have shifted to 9, 59 and 32 for males, and 17, 54 and 29 for females. Sex differences in relative tissue breadths appear to be fairly well established by 15.5 years; there is little shift

TABLE 2

Means for relative tissue breadths

AGE-LEVEL	NO.	FAT	MUSCLE	BONE
		Male (%)		
Birth	28	20	50	30
1 month	30	24	50	26
1 year	30	30	46	24
7.5 years	28	14	53	33
12.5 years	27	14	54	32
15.5 years	13	9	59	32
Adult	31	8	57	35
		Female (%)		
Birth	30	23	56	21
1 month	30	26	48	26
1 year	30	32	45	23
7.5 years	29	17	52	31
12.5 years	27	16	54	30
15.5 years	13	17	54	29
Adult	31	18	53	29

between the values at this age-level and the values in the adult, the chief difference being the increase in relative bone breadth in the males.

In table 3, mean tissue breadths are considered as ratios to mean breadths at maturity. In bone, muscle and total breadth the sexes show no great dissimilarities, and the general shape

of the patterns may be seen in figure 4 of the earlier article (Reynolds, '48). The sexes are markedly unlike in the ratios of mean fat breadth at the various age-levels to mean fat breadth at maturity.

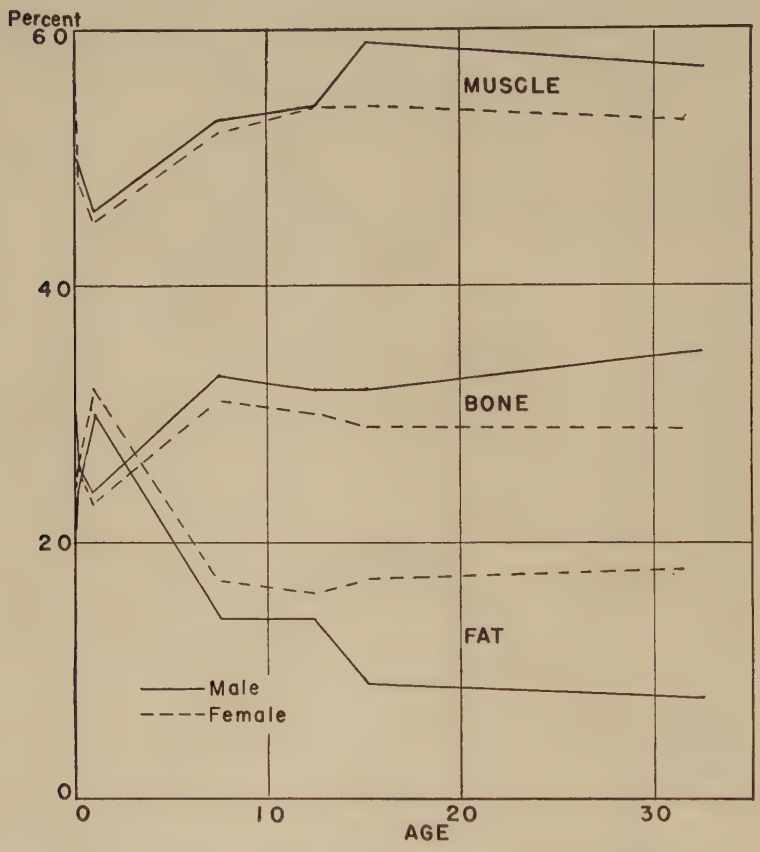

Fig. 2 Means for relative breadth of tissues, male and female.

Figure 3 shows these sex differences in fat ratios. In males, mean fat breadth at birth is 77% of the adult breadth, while the corresponding ratio for females is 43%. The ratio for males rises rapidly to 210% at one year, while the ratio for females at one year is only 109%. Thus, the absolute breadth

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of fat in the average one-year-old male, in the present series, is twice as great as the mean breadth in the adult male, while the one-year-old female infant has about the same mean fat breadth as the adult female. In males, the ratio drops during middle childhood, rises again to 147% at 12.5 years, and drops again during adolescence.

TABLE 3

Ratio of mean tissue breadths to adult means (%)

AGE-LEVEL	FAT	MUSCLE	BONE	TOTAL BREADTH
		Male		
Birth	77	28	27	31
1 month	103	31	27	36
1 year	210	46	41	58
7.5 years	118	67	68	72
12.5 years	147	84	83	89
15.5 years	114	104	94	102
Adult	100	100	100	100
		Female		
Birth	43	34	23	32
1 month	53	32	33	36
1 year	109	50	47	60
7.5 years	68	71	77	72
12.5 years	80	88	91	88
15.5 years	99	101	99	100
Adult	100	100	100	100

The sharper contrast in males between infant values and adult values is due to the much smaller breadth of fat in the adult male than in the adult female. While the mean fat breadth at one year is only 1 mm greater in females than in males, by maturity the female has on the average a breadth of fat in the calf which is 10 mm greater than the male.

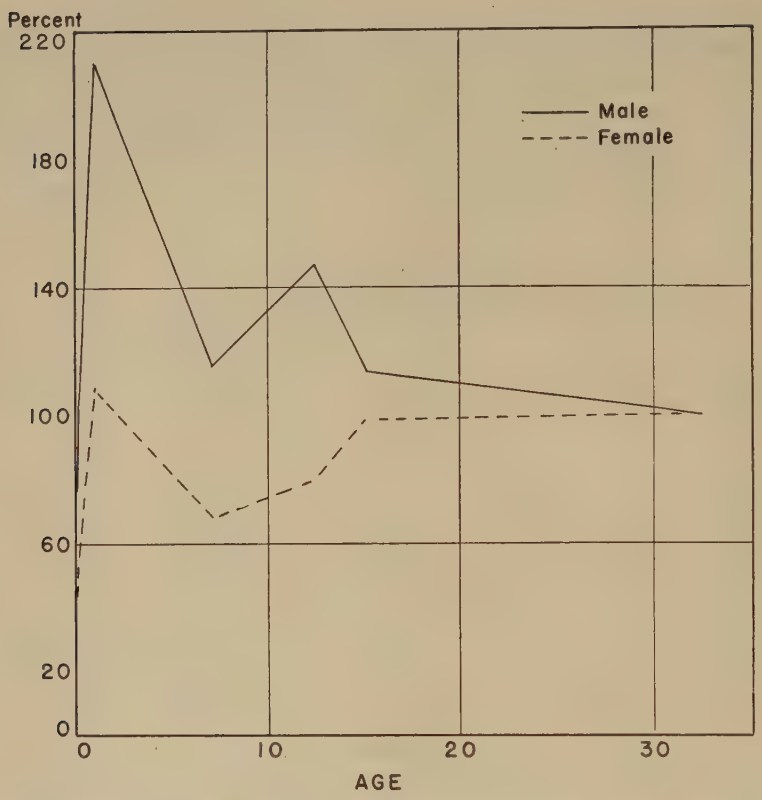

Fig. 3 Means of x-ray breadth of fat expressed as ratios of adult means, male and female.

SUMMARY

A study of tissue distribution in the male leg is compared to a previous study on females. Measurements were made of the breadth of bone, muscle mass and "fat" (tela subcutanea plus skin), as seen on anteroposterior roentgenograms of the calf, at the position of greatest breadth. Seven age-levels were studied: birth (28 cases), 1 month (30), 1 year (30), 7.5 years (28), 12.5 years (27), 15.5 years (13) and adult (31). The data are partly longitudinal. The sexes are compared with respect to mean absolute and relative tissue breadths at each age-level, and ratios of values at individual age-levels to adult size.

Males are larger than females in mean breadth of muscle, bone and total breadth of calf. Females are larger than males in mean breadth of fat. These differences are pronounced in adolescence and maturity, due to the increase in muscle breadth and decrease in fat breadth in males, and the increase in fat breadth in females. The adult female has a breadth of fat in the leg which on the average is twice as great as the adult male, and about the same as the one-year-old female. The average adult male has a breadth of fat in the leg which is half as great as the one-year-old male.

The authors wish to thank Mary Davis, of the Fels staff, for assistance in the statistical analysis.

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STAINABLE IRON DEPOSITS IN THE EPITHELIUM OF THE MAMMARY GLANDS OF MICE[1]

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THREE FIGURES

It has been pointed out (Rawlinson, '47) that depositions of stainable iron are found quite constantly in the mammary glands of female dba mice, not only in association with spontaneous adenocarcinomas, but also in precancerous stages. The latter phenomenon had been previously described but has not received much attention. It was mentioned incidentally by Gibson ('30) and Bonser ('36) but the only work that has so far been found which dealt with the matter directly is that of Schultz ('33) who described the occurrence of granules giving reactions with iron stains in the epithelial cells of the mammary glands of rats and mice. The present study confirms and extends these findings so far as mice are concerned. Mice of the dba strain from Bar Harbor were used. For histological study all the mammary glands were removed from some animals, while in others only the abdominoinguinal gland of one side was taken since, for the purpose of the study, it proved to be representative of the other glands of the same animal. The tissues were all fixed in formalin, embedded in celloidin, sectioned, and stained for iron by the potassium ferrocyanide method according to Gömöri ('36) or by the dinitrosoresorcinol method of Humphrey ('35). All the fixed tissue of each animal was completely sectioned in alternate groups of thick (100 to 200 microns) and thin (10

[1] This work was assisted by a grant from the Medical Research Fund of the University of Alberta.

to 20 microns) sections. The thick ones were stained for iron and then cleared and mounted without counterstain, and in these the accumulation of iron granules in the epithelial cells of the gland was usually great enough to outline the gland tree sharply (figs. 1 and 2). The iron varied in form from fine granules to very coarse masses scattered throughout the cells. Table 1 summarizes the observations made on the occurrence of the iron and its relation to sex and physiological states.

It can be seen from the table that the dba female mice constantly exhibited intraepithelial iron deposits in their mammary glands after reaching about 20 weeks of age. The

TABLE 1

Occurrence of histologically visible iron in the epithelium of the mammary glands

CLASS	AGE IN WEEKS	IRON	NO IRON	NUMBER OF ANIMALS
dba females: resting gland	5–19	0	28	28
dba females: resting gland	20–73	34	0	34
dba females: pregnant	13–29	3	3	6
dba females: lactating	20–38	2 (traces)	1	3
C57 Black females: rest. gld.	6–17	0	23	23
C57 Black females: rest. gld.	18–43	7	3	10

amount was decreased in some pregnant and all lactating animals, appearing in the latter either in traces only, or not at all. This confirms the observations of Schultz. Females in late stages of pregnancy had scattered tubules whose lumina contained material which stained diffusely with the iron stains, appearing as though the iron were being excreted.

The hyperplastic nodules so frequently described in the mammary glands of dba mice were also sharply outlined by virtue of their iron content (fig. 2). In the cystic dilatations commonly found in the mammary glands of old female dba mice, the lining epithelial cells usually, but not always, contained iron. Where these cells were desquamated into the lumen of the cyst, they often retained a marked iron content.

The dba males did not show iron in their rudimentary mammary glands, but they did show, with great regularity, marked deposits of stainable iron in the lymphnodes associated with the glands.

When we first made the observation of iron in the mammary glands of dba mice the possibility was considered that it might be related to the marked susceptibility of this strain to spontaneous mammary adenocarcinoma. A preliminary attempt has been made to explore this matter by establishing the presence or absence of such iron in a strain of mice of low susceptibility to mammary tumors. Mice of the C57 Black variety from Bar Harbor, having a low incidence of mammary cancer, were used and the results are also shown in table 1. As was to be expected the immature females did not show the iron granules, but at about 18 weeks of age—slightly earlier than the dba mice—they too began to show mammary gland iron (fig. 3). It appeared in this small series to be a less constant feature than in the other strain, since three animals of 30 weeks of age did not have iron.

DISCUSSION

It seems probable, since the deposits are found also in female mice of the C57 Black strain of low susceptibility to mammary cancer, that the accumulation of visible iron in the mammary epithelium of mice is not directly related to carcinogenesis, although the greater irregularity in the occurrence of iron in these preliminary experiments with the C57 Blacks invites further study. There remains the question whether the phenomenon throws light on a possible relation between the mammary glands and the apocrine sweat glands described by Richter ('32). These glands, frequently containing histological iron, are found in various locations—particularly the axilla — in many animals, including man (Bunting and Wislocki, '48, Homma '25). Schultz was impressed with the similarity between the development of the iron-containing apocrine sweat glands at puberty and the depositions of iron that occur in the mammary glands of rats and mice when they

become sexually mature. He suggested that the mammary glands of these animals act in the dual role of milk producers during lactation, and at other times as producers of sexually-active odoriferous substances. Dempsey, Bunting, and Wislocki ('47) did not find stainable iron in the mammary glands of women, female guinea pigs, cats and — contrary to Schultz — of rats. They did, however, find in two specimens of human mammary glands atypical elements resembling apocrine glands which showed a variable iron content.

One point of similarity between the apocrine glands generally and the mammary glands of mice is found in the phenomena of involution. Richter described the development in the apocrine glands with advancing age of cystic dilatations and epithelial desquamation; features of frequent occurrence in the mammary gland during involution. However, a point of difference is that the two sets of glands differ markedly in form, the branching gland tree of the mammary gland contrasting sharply with the highly convoluted but strictly tubular pattern described by Peter ('35) for the apocrine glands.

It may be, then, that there is a developmental, and perhaps even functional relationship of varying degree in different animals between the mammary glands and the apocrine sweat glands; but whatever the true explanation, the regular occurrence of such massive iron deposits in the mammary gland of the mouse merits further study from many points of view.

SUMMARY

The epithelial cells of the resting mammary gland in the mature female dba mouse constantly show stainable iron granules, frequently in massive concentrations. During pregnancy and lactation these granules decrease and may disappear. Male mice do not show them, although they do exhibit extensive iron deposits in the lymph nodes of the mammary gland regions. Mature female mice of the C57 Black strain also show iron, therefore the possibility of a direct relationship between this phenomenon and cancer susceptibility seems remote, though indications of a greater irregular-

ity in the occurrence of iron in the low cancer, as compared with the high cancer strain, calls for further study. However, the iron deposits may indicate a relationship of varying degree between the mammary glands and the apocrine sweat glands.

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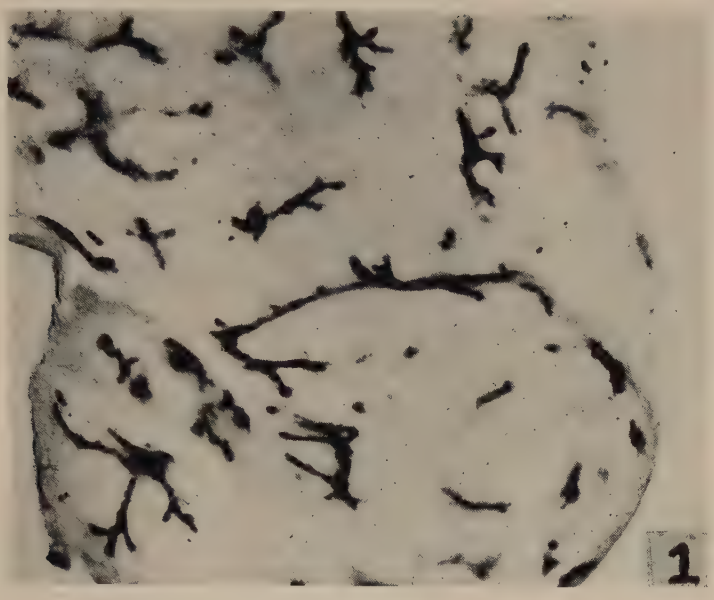
PLATE 1

EXPLANATION OF FIGURES

1 Section from resting mammary gland of female dba mouse 67 weeks of age, × 55. Section 100 microns thick, stained by Gömöri's technique for the Berlin Blue reaction, no counterstain. The iron appears deep black and outlines the gland tree.

2 Section from same source as figure 1; and similar in magnification and treatment. The stained iron outlines not only parts of the gland tree but also a hyperplastic nodule in the upper part of the figure.

3 Section from a resting mammary gland of a C57 Black female 43 weeks of age. Similar in magnification and treatment to the previous figures. The gland tree is again outlined in part by the stained iron deposits.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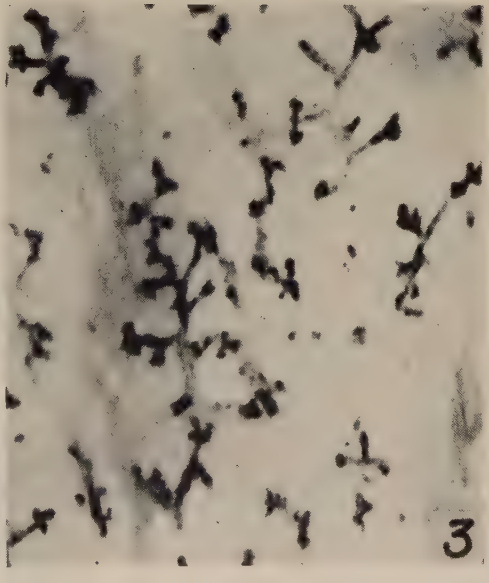
3

ORDERLY PATTERNING OF SYNAPTIC ASSOCIATIONS IN REGENERATION OF INTRACENTRAL FIBER TRACTS MEDIATING VISUOMOTOR COORDINATION[1]

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TWO FIGURES

The manner in which the integrative circuits of the nervous system became organized in development is still conjectural. For many years Kappers' ('32) electrical theory of "neurobiotaxis" was favored as the most likely explanation of how the synaptic associations become arranged in appropriate patterns. The timing of nerve discharges he supposed to be the crucial factor in determining the adaptive selectivity of neuronal interconnections. This interpretation was in accord with the widespread conviction that the refined precision, complex organization and functional adaptiveness of the underlying neural structures required for mediation of central nervous integration could hardly be attained independently of function.

A recent series of experiments on the inherent patterning of synaptic connections in the amphibian central nervous system (Sperry, '43–'48) prompted by an entirely different working hypothesis which puts no dependence upon functional factors has repeatedly yielded results consistent with the alternative view and incompatible with the neurobiotatic interpretation. In brief, without reference to the supporting evidence which has been reviewed at length elsewhere (Sperry,

[1] Aided by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

'48), this alternative hypothesis assumes that the developing neuroblasts continue to undergo a refined biochemical differentiation among themselves far beyond that which becomes manifest visibly and which closely approaches in degree the range of differentiation attained in their inherent structural associations. It is postulated further that selective affinities and incompatibilities of a physico-chemical nature develop among the various neuron types as a result of their differentiation, and that synaptic linkages are formed in a discriminative manner only between cells which have special affinity for each other. The advancing tips of the developing nerve fibers are presumed to by-pass many of the nerve cells which they encounter in their outgrowth forming synaptic endings only upon contact with particular neurons the chemical properties of which are specially matched to those of the growing fibers.

The experiments thus far have been concerned with the manner in which appropriate connections become established between center and periphery and accordingly have dealt only with synaptic formation in the primary sensory and motor nuclei. The question has therefore arisen as to whether the formation of synapses deeper in the nervous system, involving the second and higher order association neurons, might not be subject to a quite different and more functional type of regulation. It was to investigate this possibility that the following experiments were undertaken.

The observations are concerned with the reestablishment of central synaptic relations in regeneration of central association tracts following brain transection near the caudal end of the mesencephalon. Although regeneration of many different fiber tracts, both descending and ascending, was included, attention was concentrated primarily upon the descending tracts mediating visuomotor coordination. These tracts were singled out because the differential adjustment and organization of the visual functions are comparatively easy to measure, and also because the factor of functional adaptation in recovery can be readily tested in the case of vision by surgically ro-

tating or inverting the eyes or by cross-uniting the optic nerves.

EXPERIMENTAL PLAN AND METHODS

All the descending intracentral fiber tracts which link the primary visual centers with the medulla and spinal cord were severed by transecting the brain posterior to the optic lobes. The object was to find out if the divided fibers would regenerate and restore functional associations with the lower centers of the spinal cord and medulla, and, if so, whether the central synaptic associations would be restored in a haphazard, indiscriminate fashion or in an orderly, selective manner. Finally, the experiments were designed, in the latter case, to determine whether the adjustment of the central connections might be dependent upon functional types of adaptation like learning or whether it was regulated by factors intrinsic to the regeneration process itself.

If the central course and termination of the regenerating fibers were determined merely by mechanical guidance, one would anticipate only confusion and disorder in the recovered function because of the inevitable admixture of regenerating fibers of different functional types. With division of all the tracts which link cord and medulla to the higher brain centers, ascending as well as descending, there would be opportunity for intermixing of fibers not only within those tracts subserving vision but also between these and many nearby tracts of various other functions. Furthermore, even if a few fibers should chance to be directed across the cut into their original channels, they would not necessarily be guided to their former terminals because the final portion of their course within the neuropil, where the synaptic connections are made, remains unchanneled. This latter factor alone would cause much confusion in fiber termination were it dependent entirely upon mechanical guidance. Any orderliness in the recovery of function under the experimental conditions would thus necessarily indicate the action of discriminative organizing agencies of some sort.

One such agency might conceivably be the "conditioning" or learning process by which adaptive synaptic connections might be reinforced and maladaptive ones eliminated on the basis of their functional effects. Surgical rotation of the eyes through 180 degrees was carried out in some of the animals during the period in which the divided central tracts were regenerating for the purpose of testing this possibility. If these animals recovered normal visuomotor responses despite the rotated posture of the eyes, it would certainly imply a functional type of adjustment. On the other hand if the original patterns of synaptic associations were restored in the lower centers, the result would be a recovery of systematically reversed visuomotor coordinations, highly maladaptive in nature (Sperry, '43–'45). In view of the detrimental functional effects in this latter case the orderliness of recovery could hardly be ascribed to any functional process of adaptation.

With both mechanical guidance and functional adjustment eliminated, the regenerating fibers would have no means of forming systematically arranged efferent relations in the lower centers if the fibers were all alike in character. Accordingly recovery of reversed vision would constitute evidence of a qualitative specificity among the regenerating fibers and would support the idea that the formation of synaptic connections is regulated by selective affinities between the regenerating fibers and lower level neurons.

The bulk of the experiments were carried out on adult water newts, *Triturus viridescens*, during the winter months from November to March. After the animals had been deeply anesthetized with ether, the ventral wall of the cranium was opened with sharp-pointed watchmakers' forceps and broken away around the junction of midbrain and medulla. After the hypophysis had been reflected rostrally the brain was transected at approximately the level indicated in figure 1, one side at a time, by repeated crushing with an extremely fine pair of forceps, the points of which had been especially

sharpened and ground to blade-like thinness. The division of the brain substance by this method was clearly visible. It was thought that separate section of each side of the brain in bilateral cases might help reduce the mixup of fibers across the median raphe. This is important because other experiments (Sperry, '45a, '48) have indicated that some neuron types, if given a chance, readily form their characteristic associations on the opposite as well as on the normal side of the brain or cord.

Transection was performed unilaterally in 10 cases and bilaterally in 19 cases, only 11 of the latter of which survived. It was found that the mortality of the bilateral cases could be greatly reduced by keeping the animals first in cold (approxi-

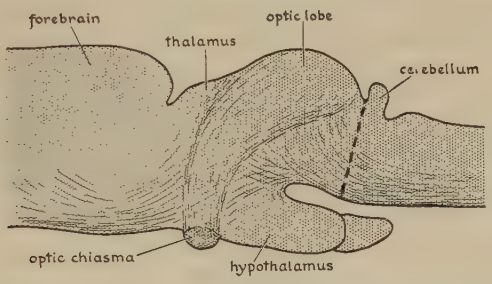

Fig. 1 The level of transection, diagrammatic.

mately 8°C.), moist containers and later in cool, shallow, well-aerated water from 4 to 8 weeks after operation or until they had reached an advanced stage of recovery. In 7 of the bilateral cases both eyes were rotated 180 degrees on the optic axis approximately 26 days after brain transection and at least 15 days before any signs of visual recovery. Records of the individual cases were kept separately, the animals being easily distinguished by their pigmentation patterns.

Optokinetic responses elicited by rotating a striped drum around the animals on the three primary axes of the body and localization and pursuit responses elicited by moving a small lure in the field of vision served as the principal criteria of visual performance. The lure consisted of a piece of dark brown gum rubber 3 mm in diameter and was kept within a

range of approximately 10 cm. After the tests of functional recovery had been completed in the animals with rotated eyes, the eyes were surgically reset in their normal orientation and the tests repeated. The results of the latter are included below along with those of the cases which retained the normal eye posture throughout. After the functional observations had been completed, the brains were prepared for microscopic examination by the Bodian silver proteinate method.

RESULTS

Immediate effects of transection. In the unilateral cases the optokinetic responses toward the side opposite the hemisection tended to be normal or exaggerated whereas the responses toward the other side were extremely weak or lacking. Responses to rotation of the visual field on the longitudinal and transverse body axes were distorted by the persistent tendency to lean and turn to the side opposite the lesion. The ability to localize small lure objects was markedly impaired. Both sides were usually affected to some extent with the impairment more severe on the side of the lesion in the majority of cases. Because all the optic fibers cross in the chiasma in these newts and because the optokinetic responses to the left are mediated only through the right eye and vice versa, the above results suggest that the fibers subserving these optokinetic responses are largely uncrossed at the level of section whereas those involved in the localization responses are largely crossed. In the bilateral cases all visual responses were abolished except in one animal which retained weak optokinetic reactions toward one side only, indicating that the transection had not been complete on one side. Various non-visual symptoms such as opisthotonus, rolling and veering to one side, swimming upside down, weakness, ataxia, etc. also appeared in varying degree and form but no attempt was made in the present experiment to keep any detailed record of the non-visual defects.

Effects of regeneration with eyes normally oriented. In the unilateral cases optokinetic responses toward the side of the

hemisection began to reappear at the end of the third post-operative week. These reactions rapidly became stronger and were accompanied by other signs of functional improvement during the following several weeks. Recovery was delayed approximately three to 6 weeks in the bilateral cases which had been kept at lower temperatures. The final tests of recovery were made in all cases between 95 and 110 days after operation.

In general, after regeneration, the lost functions were found to have been restored in an orderly systematic manner. In the unilateral cases the optokinetic responses in all three primary planes were restored to approximately their pre-operative status. The ability to localize small objects in space also underwent great improvement until no conspicuous defects remained on either side. The best cases were indistinguishable from normal with the tests employed. The bilateral cases recovered optokinetic responses correctly directed in all three planes. Responses in the frontal plane were roughly normal in character. Those in the transverse and sagittal planes, however, which normally are less pronounced, were noticeably weak in most cases. In one case the responses seemed to be lacking entirely in the transverse plane and in another no reliable optokinetic reactions could be elicited in either vertical plane.

The localizing reactions of all but three of the bilateral cases were made consistently toward the proper quadrant of the visual field for all quadrants on both sides after the eyes had been returned to normal position. There was a range in the degree of recovery, however, from the best case which responded with roughly the normal speed and accuracy to the worst cases which were obviously slower to react and less certain of the precise direction and distance of the lure. In one of the three exceptional cases no localizing responses could be elicited on one side. In the second exception, distinct erroneous reactions toward the corresponding quadrant of the visual field on the contralateral side were made consistently when the lure was presented in the dorsal quadrants on either

side. This was the animal in which the vertical optokinetic reactions were lacking. Apparently regenerating fibers which should have crossed the midline had failed to do so, or vice versa. The third exception frequently displayed crossed reactions similar to those of the preceding case when the lure was presented in the dorsal quadrant of the left visual field.

The tests gave no information on visual acuity and it is probable that acuity was quite low after regeneration. Nor did the tests furnish an exact measure of directional precision beyond that of gross quadrant discrimination. One got the impression that the responses of most of the animals, particularly the bilateral cases, were not aimed as precisely after regeneration as before. Nevertheless, correct optokinetic response to movement of the visual field around the three main axes of the body and accurate localization even though it was merely to the extent of turning to the proper quadrant of the visual field, require together a high degree of selectivity and order in the underlying neural mechanisms.

The non-visual functions likewise appeared to have undergone an orderly recovery. The various abnormalities of posture and movement had almost completely disappeared in the unilateral cases and had very largely disappeared in the bilateral group at the time of the final tests.

Recovery with eyes rotated. When visual responses reappeared in the 7 animals in which the eyes had been rotated, they showed the same systematic reversal observed after similar rotation of the eyes in animals otherwise normal (Sperry, '43a). Reversal of the optokinetic responses in the horizontal and transverse planes, spontaneous optokinetic circling and swaying movements of the head, most pronounced in the horizontal plane, and erroneous localization of small objects all appeared in characteristic fashion to a degree paralleling the subsequent display of normal reactions already described above. These maladaptive coordinations persisted without correction longer than a month in all cases until the eyes were reset in the normal orientation.

Effect of optic lobe lesions. It was possible that regeneration had taken place at random and that by "induction" effects via the efferent connections a reorganization of synaptic relations had occurred in the higher centers to suit the new patterns of connections at lower levels. There was no indication of this in the functional results of destruction of the median part of the optic lobes in 5 of the bilateral cases after the eyes had been reset. The effects of these lesions were found to be similar to those obtained in normal cases, namely, an elimination of all responses to the lure when it was presented

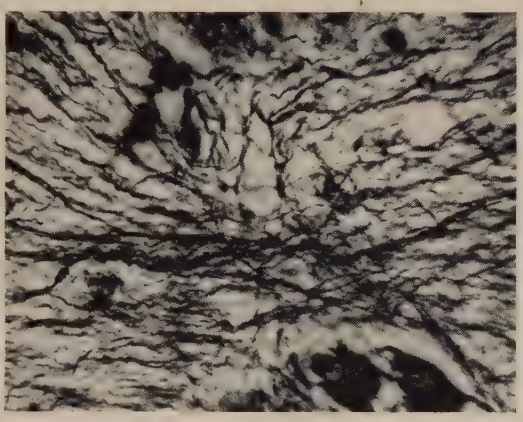

Fig. 2 Photomicrograph of a parasagittal section through the scar region showing the deflection and intermixing of the regenerated fibers despite which there occurred an orderly recovery of function. (475 ×.)

anywhere in the dorsal part of the visual field plus retention of normal reactions to the lure when it was presented in the ventral portions of the visual field. Had there been a functional readjustment in the optic lobe to suit random connections in the lower centers, the above operation should have impaired vision in the ventral as well as in the dorsal quadrants and also might possibly have left some residual vision in the dorsal quadrants. Apparently, however, the afferent system had retained its original organization and the efferent relations had been adjusted accordingly.

Histological checks. Microscopic examination of the sectioned brains revealed a copious supply of regenerated fibers crossing the gap where the transection had been made. The amount of distortion visible in the fascicular and intrafascicular fiber patterns varied in degree from two cases in which it was difficult to locate the scar line to others in which extreme distortion was evident. The photomicrograph (fig. 2) illustrates a case in which the deflection of fibers was marked but which nevertheless had exhibited an orderly recovery of function. The erratic intertangled course taken by the fibers through the scar in the majority of cases amply confirmed the inference that the methodical character of functional recovery could not possibly be ascribed to mechanical guidance of fibers across the lesion into their former pathways.

DISCUSSION

The high degree of orderliness with which functional relationships between the primary visual and lower-level centers were restored by regeneration following brain transection in the foregoing experiments cannot be accounted for by mere mechanical guidance of the regenerating fibers. The results definitely indicate the action of systematic discriminatory agencies of some kind in the recovery process. That learning or any adaptation through functional adjustment might be responsible has been ruled out by the fact that the central associations were restored in the same systematic manner even though the eyes had been previously rotated 180 degrees making the functional results of recovery highly maladaptive.

The results are explicable on the assumption that the regenerating fibers differ in character in accordance with their different afferent associations and that this constitutional specificity was able to influence in a selective manner the type of functional associations which were formed in the efferent centers. It must be concluded that the lower-level neurons likewise differ qualitatively among themselves, for, if they were all alike, there would have been no basis on which the regenerating fibers could have established associations selec-

tively. Presumably the formation of synaptic connections in the spinal and medullary centers is regulated by differential affinities between the various types of regenerating fibers and the different kinds of lower-level neurons. The regenerating fibers in their descent into the cord may be pictured as giving off numerous collaterals which arborize profusely in the central grey and superficial neuropil zones encountering many types of nerve cell bodies, dendrites, axons, glia cells, and capillaries with only a few of these contacts, limited to specific neuron types, resulting in the formation of synaptic endings.

It had already been inferred indirectly from the results of regeneration of spinal limb nerves and of the IInd, IIIrd, Vth, VIIth, and VIIIth cranial nerves (Sperry, '48) that the secondary central neurons connected with these various nerves must be qualitatively specified. The present results are entirely in accord with those obtained on synaptic formation within the primary nuclei and lend additional support to the "biochemical affinity" hypothesis of synaptic organization outlined above.

The only other possible basis of interpretation recognizable at present lies in the "resonance" and "specific nerve energy" concepts formulated by Hering ('13) and elaborated more recently by Weiss ('36, '41). It could be supposed that the visuomotor coordination of these animals is independent of selectivity among neuronal interconnections and is regulated instead through the emission of specific frequencies or modes of excitatory energies plus selective sensitization to these on the part of the different neuron types. Orderly recovery of function might be possible on this basis despite a diffuse and indiscriminate reestablishment of synaptic connections. Reasons discussed elsewhere (Sperry, '48) for continuing at this time to favor the connectionist interpretation are applicable to the present observations.

The misdirection of localizing responses toward the corresponding quadrant of the contralateral visual field clearly detectable in two of the bilateral cases indicated that the fibers of these intracentral neurons, like those of the optic

nerve and spinal sensory roots (Sperry, '45a; '48) are able to form their characteristic pattern of synaptic connections symmetrically on either side of the neuraxis. Since such connections on the wrong side of the brain or cord are highly atypical and maladaptive from the functional aspect, this is another indication that the patterning of these synaptic relations is not regulated on a functional basis. The fact that fibers became misrouted sufficiently to terminate in the wrong side of the cord also provides further evidence that there was ample opportunity mechanically for extensive intermixing of individual fiber types.

SUMMARY

When the intracentral fiber tracts subserving visuomotor coordination were transected in adult newts slightly posterior to the caudal border of the mid-brain, the divided fibers regenerated and reestablished functional relations with the lower bulbar and spinal centers in an orderly systematic manner. Mechanical guidance of the regenerating fibers and adaptation by function were both eliminated as factors responsible for the orderliness of recovery. It is suggested that differential biochemical affinities exist between the higher and lower level neurons and that the selective patterning of synaptic connections is regulated by these affinities in accordance with a modernized chemotactic interpretation of the developmental organization of neuronal interconnections.

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MESOTHELIAL CONCRETIONS AND MESOTHELIUM IN THE HUMAN PITUITARY[1]

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SIXTEEN FIGURES

INTRODUCTION

Erdheim ('04) described concretions in the follicles of the pars anterior and in and about the capsule of the human pituitary. Kraus ('12) merely mentioned that he frequently saw calcified concretions in the anterior lobe of embryos and young children. Lucien and Parisot ('14) briefly referred to calcified concretions in the anterior lobe. Plaut and Galenson ('44) clearly differentiated between concretions located in follicles of the anterior lobe and those associated with the capsule.

Rasmussen ('33) examined 235 adult human pituitaries for concretions and found them in 37% males and 71% females. He found them almost invariably located in the pia on the upper aspect of the gland near the attachment of the stalk, within and along the stalk. Examination of Rasmussen's figures shows his concretions are the same as the mesothelial concretions in our material. Shanklin ('46a) found

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mesothelial concretions in 4 of the 8 adult female and in 5 of the 12 adult male pituitaries examined. None were observed in 7 specimens from the newborn and up to 15 years.

Cushing and Weed ('15) and Weed ('20) showed that areas of hyperplastic mesothelium gave origin to the concretions found in the meninges of the brain. Shanklin ('46a) described the development of the concretions, in and about the capsule of the human pituitary, from mesothelium. He also showed that in the development of these concretions one or more mesothelial cells became hypertrophied, these were then surrounded by other mesothelial cells which formed a membrane around the enlarged cell. Following this a series of lamellae were formed and each was surrounded in turn by interlamellar membranes formed by the peripherally placed mesothelial cells. The concretion was frequently surrounded by a capsule of densely packed collagenous fibers. These concretions were often observed undergoing degeneration, a process described by the writer as "melting down."

Islets of epithelium or "Plattenepithelinseln" were described in the human pituitary by Erdheim ('04), Christeller ('14), Priesel ('22) and Kiyono ('24). Erdheim reported finding plates of epithelium in 10 of 13 pituitaries examined, Christeller 20 out of 34 and Priesel 3 out of 20. Kiyono found these plates in 17 of 50 pituitaries examined, but none in the 9 cases under 20 years of age. He observed them most frequently in the anterior part of the stalk.

The writer proposes the term mesothelial concretions for those associated with the capsule and derived from mesothelium and the term follicular concretions for those found in the anterior lobe follicles. The writer wishes to emphasize that the epithelium in the epithelial plates of Kiyono and earlier investigators was not differentiated by them into the two major varieties found there; mesothelium and squamous epithelium. Only the mesothelial concretions and mesothelium are described in this paper.

It is our purpose to describe the incidence, exact location and histology of the mesothelial concretions and mesothelium in 100 human pituitaries.

MATERIALS AND METHODS

This paper is based on a study of 100 human pituitaries all of which were obtained from autopsy and sectioned serially at 10 μ. The stain most frequently used was hematoxylin and eosin. Each of the serial sections (about 500 per adult pituitary) was systematically examined for mesothelial concretions and mesothelium. Part of this material was used by the writer for earlier studies (Shanklin, '46a, '46b and '47).

Our departmental number, the sex, age, cause of death and a summary of the histological findings are given in table 1. Although only mesothelial concretions and mesothelium are dealt with in this paper, the writer proposes to describe the other histological components such as squamous epithelium, glands, adult tumorettes and lymphocytes in subsequent papers.

OBSERVATIONS ON MESOTHELIAL CONCRETIONS

Incidence and sex ratio (tables 1 and 2). The youngest pituitary in our series with mesothelial concretions was a 4-year-old male (M107) and the oldest was an 86-year-old male (M233). Of the total 100 pituitaries examined 71 were from persons between 4 and 86 years. The remaining 29 include the newborn and up to three years. Of the 71 pituitaries from individuals between ages 4 and 86 there were 25 from females and 46 from males.

Mesothelial concretions were observed in 51 specimens (71.8%) of the 71 persons between the ages of 4 and 86. The concretions were found in 18 (72.0%) of the 25 females and in 33 (71.7%) of the 46 males.

Another analysis of the data was made to determine whether or not, concretions occur more frequently in old age. As the preceding analysis failed to suggest a significant sex difference in the occurrence of these concretions; pituitaries

TABLE 1

Table giving summary of histological findings in 100 human pituitaries, including age, sex and cause of death

NO. OF SPECIMEN	AGE AND SEX	MESOTHELIAL CONCRETIONS	MESOTHELIUM	GLANDS	TUMORETTES	FIBROID NEUROGLIA	LYMPHOCYTES	CAUSE OF DEATH
M86	F, 45 [1]	+	+	+	+			Acoustic neuroma
M88	M, 15			+			+	Auto. accident
M89	M, 40	+					+	Suicide
M91	M, 40	+	+				+	Suicide by hanging
M92	M, 65	+	+			+	+	Suicide by lysol
M95	F, 59	+	+		+			Brain tumor
M98	F, 80							Hernia, artheroma of aorta
M100	M, 65	+	+		+		+	Suicide
M101	M, 37	+	+	+		+	+	Brain tumor
M106	M, 42	+	+		+	+	+	Brain tumor
M107	M, 4	+	+	+			+	Brain tumor
M108	M, 1			+				?
M109	M, 43	+	+	+		+	+	Brain tumor
M110	F, 3.5					+	+	Brain abscess
M111	F, 30	+	+	+		+		Carcinoma of breast
M112	M, 51	+	+	+	+	+	+	Brain tumor
M113	M, 7							Brain tumor
M114	M, 33	+		+		+	+	Aneurysm of cerebral artery
M115	M, 40	+	+	+	+		+	Progressive paralysis
M116	M, 65			+			+	Shock
M117	F, 52	+	+	+				Brain tumor
M118	F, 14	+	+			+	+	Brain tumor
M119	F, 31	+	+	+			+	Carcinoma of breast
M120	F, 1.5			+				Diphtheria
M121	M, newborn			+				Asphyxia
M122	F, 11	+	+	+			+	Brain tumor
M123	M, 70	+	+					Burns
M124	F, 20	+		+			+	Burns
M125	M, 53	+	+	+	+		+	Brain hemorr.
M126	M, 12 da.			+				Pneumonia
M163	M, 3.5 mos.			+				Poisoning
M164	M, 52	+	+	+		+		Traffic accident
M165	M, 1 mo.			+				Pyelonephritis
M166	M, 1			+				Pneumonia
M167	M, newborn			+				Asphyxia
M168	F, 18		+	+		+		Encephalitis?
M169	M, 48					+		Brain hemorr.
M170	M, 65	+	+				+	Atrophy of brain
M171	F, 18	+	+			+	+	Endocarditis
M172	F, 83	+	+	+	+		+	Thrombosis of cerebral veins
M173	M, 8 mos.			+				Asphyxia
M174	M, 69	+	+	+		+		Occlusion coronary artery
M175	M, 10 mos.			+				Bronchitis
M176	M, 70	+	+			+		Aneurysm of aorta
M177	M, 35	+	+			+		Coronary atheroma
M178	F, 72	+	+			+	+	Cardiac dilation
M179	M, 7 mos.			+				Otitis media
M180	M, 4.5			+				Brain tumor
M181	F, 1 day			+				Atelectasis

TABLE 1 (*continued*)

Table giving summary of histological findings in 100 human pituitaries, including age, sex and cause of death

NO. OF SPECIMEN	AGE AND SEX	MESOTHELIAL CONCRETIONS	MESOTHELIUM	GLANDS	TUMORETTES	FIBROID NEUROGLIA	LYMPHOCYTES	CAUSE OF DEATH
M182	F, 6							Poisoning
M183	F, 39	+	+		+			Ovarian carcinoma
M184	M, 5 days			+				Pneumonia
M185	M, 14 days			+				Brain abscess?
M186	F, 6 mos.			+				Cirrhosis of liver
M187	F, 6			+				Tuberculosis
M188	F, 36	+		+				Brain abscess
M190	F, 4 days			+				Subdural hematoma
M192	M, 3			+				Poisoning
M193	F, 3.5							Diphtheria of skin
M194	M, 9 wks.			+				Meningitis?
M195	F, 6 mos.			+				Meningitis
M196	M, newborn			+				Hematoma
M197	F, 1.6			+				Suffocation
M198	F, newborn							Asphyxia
M199	M, 22	+	+	+			+	Brain abscess
M200	M, 2 days			+				Subdural hematoma
M201	M, newborn			+				Subdural hematoma
M202	F, 3 mos.							Brain abscess
M204	M, 40	+	+	+	+		+	Brain tumor
M205	M, 35			+	+	+	+	Endocarditis
M206	M, 40	+	+					Subarachnoid hemorr.
M207	M, 48	+	+	+			+	Embolus of basilar artery
M208	M, 2 days			+				Asphyxia
M209	M, 20	+	+	+	+	+	+	Cerebral embolus
M210	M, 60	+	+		+		+	Pellagra
M213	F, 41	+	+					Acoustic neuroma
M214	M, 16		+	+			+	Suicide by shooting
M215	F, 12	+	+					?
M217	M, 40				+			Suicide, poison
M218	F, 17			+			+	Embolism
M220	M, 43		+	+		+	+	Brain tumor
M221	M, 15			+			+	Endocarditis
M223	M, 49	+			+	+		Kidney abscess
M224	F, 52	+	+	+		+	+	Brain tumor
M226	M, 45	+	+	+			+	Brain tumor
M227	F, 21	+	+	+		+	+	Sarcoma
M228	M, 48		+		+		+	Burns
M229	M, 23	+	+				+	Brain tumor
M230	M, 35	+	+				+	Brain abscess
M232	M, 40	+	+				+	Brain hemorr.
M233	M, 86	+	+					Brain hemorr.
M235	M, 45	+	+	+				Aneurysm, brain
M238	F, 4			+			+	Bullet in brain
M239	M, 13	+	+	+			+	Brain hemorr.
M240	F, 19			+			+	Bullet in abdomen
M242	M, 45		+	+	+	+		Parapharyngeal abscess
M244	F, 36	+	+	+		+		Carcinoma of uterus
M245	M, 4							Cerebral vein thrombi
M246	M, 20	+	+					Typhoid fever
M248	F, 3.5			+			+	Otitis media

[1] Islets of squamous epithelium were seen in 6 specimens; M91, M115, M217, M224, M229, M233.

TABLE 2

Number and location of mesothelial concretions in the pituitary

NO. OF SPECIMEN	SEX AND AGE	CAPSULE ZONE OF INSERTION	CAPSULE PARS ANTERIOR	CAPSULE PARS NERVOSA	CAPSULE STALK	BETWEEN STALK AND PARS ANTERIOR	DEEP IN PARS ANTERIOR	DEEP IN PARS NERVOSA	TOTAL CONCRETIONS	AMOUNT OF MESOTHELIUM [1]
M86	F, 45	1							1	Scanty
M89	M, 40		1						1	None
M91	M, 40	2	1	4	4			1	12	Medium
M92	M, 65	12	66		4		1		83	Abundant
M95	F, 59	10	5			10	36		61	Abundant
M100	M, 65	3							3	Scanty
M101	M, 37	3							3	Scanty
M106	M, 42			3					3	Abundant
M107	M, 4	32	30	4					66	Medium
M109	M, 43		5		2			1	8	Scanty
M111	F, 30							1	1	Scanty
M112	M, 51	1	2						3	Abundant
M114	M, 33	11	6						17	None
M115	M, 40	9	2	3	1			1	16	Scanty
M117	F, 52		3			31			34	Medium
M118	F, 14	8							8	Medium
M119	F, 31		5						5	Abundant
M122	F, 11	4	1						5	Abundant
M123	M, 70			10					10	Abundant
M124	F, 20	111	3	7	37			2	160	Abundant
M125	M, 53	13	6	1	15				35	Abundant
M164	M, 52	94	9	24					127	Abundant
M170	M, 65		76						76	Abundant
M171	F, 18		5						5	Scanty
M172	F, 83	54	82	99	6		1		242	Abundant
M174	M, 69		3						3	Scanty
M176	M, 70	13	69	14	10			9	115	Abundant
M177	M, 35		1						1	Scanty
M178	F, 72	15	15	11	21				63	Medium
M183	F, 39	6	16	1					23	Medium
M188	F, 36	7							7	None
M199	M, 22		2						2	Medium
M204	M, 40	1		1					2	Scanty
M206	M, 40	2							2	Medium
M207	M, 48		31	2			2	8	43	Medium
M209	M, 20	3	6						9	Scanty
M210	M, 60	25	287	154					466	Abundant
M213	F, 41	1	4	3					8	Scanty
M215	F, 12		4	2					6	Scanty
M223	M, 49			34					34	None
M224	F, 52	4	62						66	Scanty
M226	M, 45	14	2	4					20	Scanty
M227	F, 21	4	10						14	Medium
M229	M, 23		4						4	Abundant
M230	M, 35	2	4						6	Scanty
M232	M, 40	26	2	14	2				44	Medium
M233	M, 86	12	12						24	Medium
M235	M, 45		2						2	Scanty
M239	M, 13		2						2	Scanty
M244 [2]	F, 36	46	9	11					121	Abundant
M246	M, 20		9						9	Scanty
Total		549	864	406	103	41	40	23	2081	
Average		10.8	16.9	8.0	2.0	.8	.8	.5	40.8	

[1] Mesothelium was also found in 5 other specimens; M168 abundant, M214 scanty, M220 abundant, M228 scanty, M242 scanty.

[2] Fifty-five in area of inflammatory cells in this pituitary.

from both males and females were grouped together in three age groups; 4 to 19, 20 to 39 and 40 to 86 years. Concretions were present in 6 (33.3%) of the 18 pituitaries in the 4 to 19 age group, in 15 (93.7%) of the 16 pituitaries from 20 to 39 and in 30 (81.1%) of the 37 pituitaries in the 40 to 86 age group.

A 3rd analysis of the data (table 2) was made to determine whether or not there was an increase in the actual number of concretions with increase in age. The average number of concretions for the total 18 specimens in the youngest group (4 to 19 years) was 5.1, for the total 16 pituitaries in the middle group (20 to 39 years) it was 23.9 and for the 37 in the oldest group (40 to 86 years) it was 43.4. Including only those pituitaries in the three age groups which had concretions the average for the youngest group was 15.3, for the middle group 25.5 and for the oldest group 53.6.

Location in pituitary (table 2). Those mesothelial concretions at the surface of the pituitary are always found associated with its upper aspect. In the table their location is given as "Capsule Zone of Insertion, Capsule Pars Anterior, Capsule Pars Nervosa and Capsule Stalk." Although many of these concretions are actually embedded in the capsule of the regions given, many others lie to the outside in the adjacent pia, while some are actually found wedged in between the inner border of the capsule and the parenchymatous elements of the anterior or of the posterior lobe. The locations of some of these are illustrated in the figures. For example, two mesothelial concretions are shown in figure 9 along the outer border of the stalk, while two others are seen (fig. 1) in a cleft of the capsule around the stalk. Multiple concretions are shown (fig. 7) associated with the capsule at the zone where the stalk joins the infundibular process. These concretions are also occasionally found lying deep in the pituitary between the stalk and the anterior lobe (fig. 8).

One pituitary had a number of typical large mesothelial concretions, in a mass of connective tissue in the center of the pars anterior. One of these is included in figure 4.

These concretions are occasionally found among the epithelial cells of the anterior lobe (figs. 5 and 6) or deep among the parenchymatous cells of the infundibular process (fig. 2). Early developmental stages of concretions are also shown (figs. 8 and 11) among areas of mesothelium. These concretions are also found associated with extensive invasion of the capsule by areas of hyperplastic mesothelium (fig. 16). A number were observed in a large area of inflammatory cells that had replaced a considerable part of the neurohypophysis. One of the latter is shown in figure 14.

Number in different locations (table 2). The line of demarkation, between the region of the capsule where the stalk is inserted, "Capsule Zone of Insertion," and the contiguous areas, is not a sharp one hence in counting the concretions it was necessary at times arbitrarily to include them under what appeared to be the most likely location. In regions where the concretions were large and relatively sparse it was easy to make accurate counts, however where there were many small closely packed ones the counts are not entirely accurate.

Table 2 includes the specimen number, sex, age, number of concretions in different parts and relative amount of mesothelium in 51 human pituitaries, ranging from 4 to 86 years.

In the total 51 pituitaries with mesothelial concretions these concretions were found most frequently (40 times) associated with the capsule of pars anterior. Next in frequency was the capsule in the region of the stalk insertion (32 times), followed by capsule of pars nervosa (21 times) and capsule of stalk (10 times). In the three remaining sites they were only occasionally found. The average number of concretions found in the different sites, 51 positive cases only included, were as follows: capsule pars anterior 16.9, capsule zone of insertion 10.8, capsule pars nervosa 8.0, capsule stalk 2.0, between stalk and pars anterior 0.8, deep in pars anterior 0.8 and deep in pars nervosa 0.5.

Development and histology. These topics were dealt with in an earlier paper (Shanklin, '46a) hence only a few additional comments are made here. The difference in staining of the

lamellae in the same concretion is due to the varying amounts of calcium in the different layers. In general the central area is relatively free of calcium hence stains more lightly. In older concretions, such as the one to the right in figure 1, where there is advanced senescence, the calcified lamellae stand out in sharp contrast to the central zone. Variations in the degree of lamellar calcification is also well shown in the writer's earlier paper on concretions (figs. 8 to 10 and 12). Rasmussen ('33) noted that the calcium in these concretions frequently nicked the microtome knife. We have had a similar experience. Due to the calcification the concretion is frequently displaced when the microtome knife strikes it. This displacement causes crushing of the adjacent tissue on the side away from the point where the knife strikes. The knife also frequently depresses the concretion unevenly so that one side is out of focus in photomicrographs. Both of these points are demonstrated in figure 2.

OBSERVATIONS ON MESOTHELIUM

Incidence. Epithelial plates in the pituitary may consist either of mesothelium or of squamous epithelium. In studying the figures and text of papers by Erdheim ('04), Christeller ('14), Priesel ('22), and Kiyono ('24) it is frequently difficult to determine which variety is referred to. The squamous epithelium, with intercellular bridges, is relatively rare in our series. As a matter of fact we only observed this variety in 6 of our 100 pituitaries (M91, M115, M217, M224, M229, M233). Squamous epithelium was only seen in the capsule of the upper aspect of the pituitary, but mesothelium was found lying within and on both sides of the capsule.

Mesothelium was found in 52 pituitaries, or 52% of the 100 examined. The youngest specimen observed with mesothelium was from the same 4-year male (M107) with numerous mesothelial concretions. Of the total 100 pituitaries 71 specimens were from individuals between 4 and 86 years. Fifty-two of the 71 pituitaries or 73.2% in this age group contained mesothelium.

Although in most cases mesothelium and mesothelial concretions were observed in the same specimen they were not always found together. Both were found in the same specimen 47 times, whereas mesothelial concretions were seen 4 times in the absence of mesothelium. Likewise mesothelium was observed 5 times in the absence of mesothelial concretions. Frequently the mesothelial concretions are embedded in or in contact with an area of mesothelium. The intimate relationship of the two is shown in figures 1, 8 and 9.

The amount of mesothelium present is indicated in table 2 as scanty, medium and abundant. Of the 52 positive specimens, 22 are recorded as scanty, 12 as medium and 18 as abundant. In general those pituitaries with no mesothelium or only a scanty amount have relatively few mesothelial concretions, whereas those with abundant mesothelium have many mesothelial concretions. For example those 23 pituitaries, with mesothelial concretions, but no or only scanty mesothelium had only 10.0 concretions on the average, while the remaining ones with medium to abundant mesothelium had on the average 66.1 concretions.

Location in pituitary. The exact location of mesothelium in the pituitary was not recorded for each specimen but the favorite sites were the same as those for the mesothelial concretions. The most frequent being in the capsule of the zone where the stalk is inserted into the pituitary. The mesothelium is often found embedded in the connective tissue of the capsule and even to the inner side of the capsule. Although it frequently lies to the inner side of the capsule as a rule it does not penetrate into the adjacent parenchymatous elements of the anterior lobe or of the neurohypophysis. Like the mesothelial concretions the mesothelium was seen occasionally wedged in between the stalk and the anterior lobe (fig. 8).

Mesothelium in the pituitary may be found as scattered cells, long thin cords, as oval to elongated plates, or large irregular shaped masses (figs. 8 to 11).

Histology. The nuclei of the mesothelial cells are usually oval or fusiform in shape, moderately stained and have finely granular chromatin which is fairly evenly distributed (fig. 10). As a rule the cytoplasm of the mesothelial cell is relatively sparse, however, in areas of hyperplasia it may increase in amount. Occasionally a single nucleus rounds up and becomes markedly increased in size (fig. 11). At the same time the normal cytoplasm of some of these cells is replaced by a pale stained material and the cell becomes greatly enlarged (fig. 11). These enlarged mesothelial cells represent the early developmental stage of the mesothelial concretions. Other mesothelial cells become closely applied to these enlarged cells and give rise to long processes which form a membrane that encircles the enlarged cell. For a full description of these early developmental stages in which mesothelium is transformed into mesothelial concretions see the writer's earlier paper ('46a). The single enlarged cell in figure 11 has two nuclei closely applied to its surface. This represents the stage before the cytoplasm of these cells form an encircling membrane.

Areas of hyperplastic mesothelium are frequently observed in the human pituitary (figs. 10, 11 and 16). The areas often have whorls of mesothelial cells (figs. 12, 13, 15 and 16).

DISCUSSION

Plaut and Galenson ('44) examined the pituitary glands of 150 newborn children and young infants for concretions. They observed them in the epithelial portions of the gland in all newborn infants and in most infants under the 6th postnatal month. They found a sharp decrease in these concretions during the 1st year of life and concluded that they decrease or disappear in early infancy. In spite of their decrease during infancy they observed some in pituitaries of persons varying from 3 to 68 years. Plaut and Galenson refer to these as colloid concretions and to those associated with the capsule of the pituitary gland as meningeal concretions. We wish to emphasize the observation of Plaut and Galenson

that these colloid concretions differ from those of the capsule; but because the latter develop from mesothelial cells (Shanklin, '46a), we propose the more specific term mesothelial concretions rather than meningeal concretions.

We found mesothelial concretions in almost the same percentage of males (71.7) as in females (72.0). The percentages given by Rasmussen ('33), based on 235 adult pituitaries, were 37 for males and 71 for females. In our earlier study on the development of concretions, based only on 20 specimens, we found mesothelial concretions in 41.7% of males and 50.0% of females. Our larger sample shows almost exactly the same percentage of positive cases in females as found by Rasmussen, however, our positive findings in the male are considerably higher. The slightly higher total percentage of positive cases in our series, as compared with that of Rasmussen, can be explained by the fact that we examined each section, whereas Rasmussen examined only every 20th section. As we only found from one to three concretions in 14 of the pituitaries examined it is very likely that Rasmussen failed to find all the positive cases.

No mesothelial concretions were observed in the 29 pituitaries from the newborn and infants one to three years old. Our youngest positive pituitary was specimen (M107) from a 4-year male. This pituitary had 66 concretions. The other 5 positive cases in the younger group were 11, 12, 13, 14 and 18 years. Rasmussen ('33) included material only from individuals 19 years and older. Our material proves that these concretions do occur in children even as young as 4 years. Krabbe ('16) found concretions (acervuli) in the pineal gland of a 1.5-year-old child but was of the opinion they usually begin to occur at the age of 14.

We shall attempt to answer two questions regarding the occurrence of these concretions. First, is the incidence higher in older pituitaries? Second, do they tend to increase in numbers during the process of ageing? Rasmussen found no difference in the incidence of concretions in persons under and over 50 years of age, nor in pregnant women. In our

series pituitaries with mesothelial concretions were found less frequently (33.3%) in individuals between 4 and 19 years, most frequently (93.7%) between 20 and 39 years. The oldest age groups (40 to 86 years) had fewer positive cases (81.1%). Our study shows that mesothelial concretions occur less frequently in children, but, like Rasmussen, we failed to find an increase in persons with concretions in the older age groups. Our material suggests, however, that the actual number of concretions in the positive cases do increase with age. Including only those 51 pituitaries with concretions we found an average of 5.1 concretions per pituitary in the youngest group (4 to 19 years), 23.9 in the middle group (20 to 39 years) and 43.4 in the oldest group (40 to 86 years).

Rasmussen found these concretions almost invariably situated in the pia on the upper aspect of the gland near the attachment of the stalk, both within and along the stalk. In general our observations agree with those of Rasmussen. Frequently we found them deep in the capsule and at times actually between the inner border of the capsule and the parenchymatous elements of the anterior lobe, the posterior lobe, or the stalk. Some of those included in the figures lie deep in the pars anterior and in the infundibular process. No concretions were observed in or about the capsule of the pituitary on the side opposite to the stalk. Examination of table 2 shows their average number in the various locations. They are most frequently associated with the capsule of the pars anterior, next with the capsule where the stalk is attached to the pituitary, followed by capsule of pars nervosa and capsule of stalk. Only occasionally were concretions observed deep in the pituitary.

In the earlier papers dealing with epithelial islets or plates in the human pituitary the variety of epithelium observed is usually not clearly stated. In the excellent paper by Kiyono ('24) on the occurrence of epithelial plates in the hypophysis some of his figures 1, 2, 7 and 9 show areas that look like mesothelium while in his figure 8 the cells can be readily identified as simple squamous.

Erdheim ('04) found epithelial plates in 76.9% of 13 pituitaries examined, Christeller ('14), in 58.8% of 34 specimens, Priesel ('22) in 15.0% of 20 specimens and Kiyono ('24) in 34.0% of a series of 50. Kiyono failed to find epithelial plates in 9 cases under 20 years.

We found an incidence of 73.3% for mesothelium in our series of 71 human pituitaries from individuals between the ages of 4 and 86 years. This frequency is much higher than that of Christeller and Kiyono, however, it can be easily accounted for by the fact that in our series every section was examined.

Erdheim ('04) failed to find epithelial plates in the 7 foetal and newborn pituitaries examined, however, he thinks they are present but very small. Christeller ('14) found them in a 4-year-old child. The youngest specimen in our series containing mesothelium was from the same 4-year child (M107) in which we found mesothelial concretions.

The fact that these concretions develop from the arachnoidal mesothelium (Shanklin, '46a) would lead us to expect to find the two in the same areas. In general this is true for they are usually found together. The presence of the mesothelium and mesothelial concretions around the stalk and the upper aspect of the pituitary can be explained by the anatomical relationship of the meninges to the pituitary. Sunderland ('45), in a study of 7 infants and 26 adults, found a rather large diaphragma sellae only partly occupied by the infundibulum and pars tuberalis with the residual lumen closed and the fossa completely sealed off by the reflection of the pia from these structures to the margins of the aperture. He says that in infancy and childhood there is normally no arachnoid membrane below the level of the dural aperture, owing to the closure by the pia and the gland. In pituitaries from old individuals he found the gland sunk into the fossa, thereby enabling the pia, the arachnoid and the subarachnoid space to descend for varying distances over the upper surface of the gland. According to Sunderland's description of the meninges, the mesothelium and its derivatives, the mesothelial

concretions, should be limited chiefly to the region of the stalk and the upper aspect of the pituitary.

The presence of mesothelium and mesothelial concretions in the substance of the capsule and even along its inner border can be explained by the ability of the mesothelium to penetrate into any small cleft between the connective tissue fibers. Frequently the small clefts through which an area of mesothelium has invaded the capsule can still be seen in the sections. Earlier studies by Cushing and Weed ('15, figs. 2 and 7), Weed ('20, figs. 11 and 12) and by Weed ('32, fig. 6) give examples of the mesothelial cells penetrating into the connective tissue elements of the dura.

We are of the opinion that those mesothelial concretions deep in the substance of the anterior and the posterior lobe were formed by mesothelium that migrated into those areas with the blood vessels and with the expanding connective tissue elements. We have no exact knowledge regarding the time at which the vascular system in the human pituitary expands, however, the writer (Shanklin, '44, fig. 7) has shown that there is an extensive spreading of the capillaries in the neurohypophysis in the 180-mm pig. A similar expansion of the blood vessels in the foetal human neurohypophysis was not observed (Shanklin, '40) but this aspect of the problem needs further study in man. Erdheim ('04) found concretions in two cases in the intermediate vascular connective tissue strand which traverses the anterior lobe. He said that the arachnoidea of the newborn goes up to the infundibulum and from there extends an arachnoidal fold whose lower end lies in the angle between the hypophysis and its stalk. According to this description the arachnoidal mesothelium, or at least its precursors are near the upper aspect of the human pituitary at the time of birth, hence might be carried in by the invading blood vessels.

Erdheim ('04) and Kiyono ('24) each found islets of squamous cells, most of which according to our interpretation are mesothelium, in a single pituitary wedged in between the anterior and posterior lobes. We also found numerous meso-

thelial concretions and abundant mesothelium in a similar situation in a 52-year female (M117) and in a 59-year female (M95). This peculiar location of the mesothelium and concretions finds its explanation in a study of the variations in the place of insertion of the stalk and in the developmental history of the pars anterior. Erdheim has shown that the exact place of insertion of the stalk is variable. For example, in his figures 3 and 5 the stalk is inserted approximately in the middle of the upper hypophyseal surface, while in figures 2 and 4 it is inserted more posteriorly and in figure 6 more anteriorly. Erdheim says that most frequently the stalk is inserted more posteriorly running in a shallow groove along the posterior surface of the anterior lobe in order to reach the posterior lobe, however, in those cases where it is inserted more anteriorly it has to perforate the tissue of the anterior lobe in order to reach the posterior lobe. He says furthermore that in speaking of perforation of the anterior lobe by the infundibulum we are using the term in a purely anatomical sense, not developmentally for the embryological process is actually one of encirclement of the infundibulum by the growth of the anterior lobe. Our findings are in full accord with those of Erdheim and we believe that some of the mesothelium along the anterior aspect of the stalk is surrounded by the overgrowth of the anterior lobe. At a later period the mesothelium in that region gives rise to the mesothelial concretions in the area.

Our material shows very clearly the close correlation between the presence of mesothelium and mesothelial concretions in the same pituitary for they were both found in the same specimen 48 times. Mesothelial concretions were seen only 4 times without mesothelium. This may have been due to the smallness of the area, or the dispersed state of the mesothelial cells. The occurrence of mesothelium without concretions is not surprising as areas of it are frequently seen in the pituitary and in the meninges without concretions. Analysis of our data showed that in general those pituitaries

with scanty mesothelium had fewer concretions while those with abundant mesothelium had numerous concretions.

We wish to emphasize the similarity between the mesothelium and mesothelial concretions of the pituitary with those of the meninges. For example, the areas of hyperplastic mesothelium in the capsule of our pituitary (fig. 10) is very similar to that shown by Weed ('32, figs. 6, 9 and 10). Again the similarity is seen in the whorls in our material (figs. 12, 13, 15 and 16) and those shown by Cushing and Weed ('15, figs. 7 A and 7 B), and by Weed ('20, figs. 5 to 12). Similarly the mesothelial concretions lying among mesothelial cells shown in our figures 1, 8 and 9 are like those of Cushing and Weed ('15, fig. 7 B) and those of Weed ('20, figs. 7 to 11).

Erdheim ('04) and Kiyono ('24) both thought that the epithelium in the epithelial plates of the pituitary were derivatives of Rathke's pouch. Erdheim ('26) even refers to it on p. 500 as "Mundepithels." We believe that the epithelium in the epithelial plates described by Erdheim, Kiyono and others consists of two varieties, one squamous epithelium with intercellular bridges, the other mesothelium. The first variety is relatively rare for we only found it in 6 of the 100 pituitaries examined, while the mesothelium occurs frequently. The occasional area of squamous epithelium may be a derivative of Rathke's pouch but certainly the more numerous areas of mesothelium come from the arachnoidal mesothelium. For a full account of the arachnoidal mesothelium the reader is referred to the description by Weed ('32).

Conclusion. We conclude on the basis of a study of 100 specimens that both the mesothelium and its derivative, the mesothelial concretions, appear in the human pituitary about the age of 4 years. No difference was found in the incidence of these concretions in the two sexes. In general those pituitaries with abundant mesothelium had numerous concretions. Although the number of pituitaries with concretions did not increase in the older age groups, there was a definite increase in the number of concretions per positive pituitary. Both mesothelium and mesothelial concretions were found chiefly

associated with the capsule of the upper aspect of the pituitary, however, a few were observed deep in the anterior and the infundibular process. The presence of mesothelium and mesothelial concretions deep in the pituitary is explained on an embryological basis.

SUMMARY

1. Complete serial sections of 100 human pituitaries obtained from individuals between the newborn and 86 years of age were systematically examined for mesothlium and mesothelial concretions.

2. The youngest specimen in which mesothelium and mesothelial concretions were observed was from a boy 4 years old.

3. No difference was found in the incidence of mesothelial concretions in the two sexes.

4. In general those pituitaries with abundant mesothelium had numerous concretions.

5. The number of pituitaries with concretions did not increase after the age of 20, however, in those pituitaries where they were present the concretions were more numerous in old age.

6. The presence of mesothelium and mesothelial concretions chiefly about the upper aspect of the pituitary is explained on an anatomical basis, however, their presence deep in the pituitary is given an embryological interpretation.

7. According to our interpretation most of the pituitary epithelial islets of earlier writers are areas of mesothelium which are derived from the meninges and are not derivatives of Rathke's pouch.

8. The similarity between the areas of mesothelium and the mesothelial concretions of the pituitary and those of the meninges is stressed.

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PLATE 1

EXPLANATION OF FIGURES

All photomicrographs are of sections from the human pituitary and are stained with hematoxylin and eosin.

1 Two mesothelial concretions and mesothelium in the capsule of the stalk. × 383.

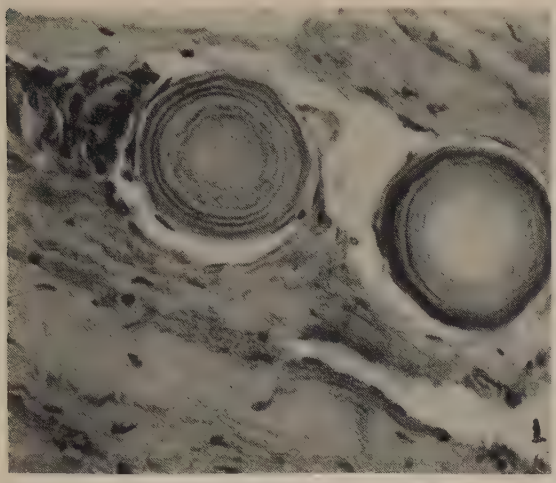
2 A mesothelial concretion in the infundibular process. × 234.

3 A mesothelial concretion located between the capsule and epithelium of pars anterior. × 234.

4 A double mesothelial concretion in the connective tissue in center of pars anterior. × 234.

5 A mesothelial concretion situated in a crevice in pars anterior. × 383.

6 A mesothelial concretion in the pars anterior. × 383.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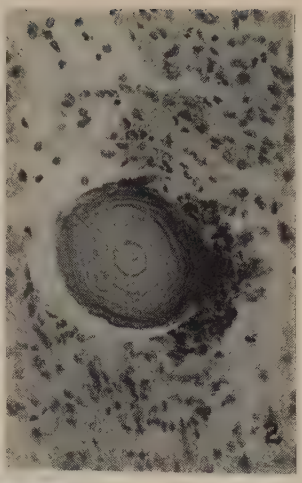
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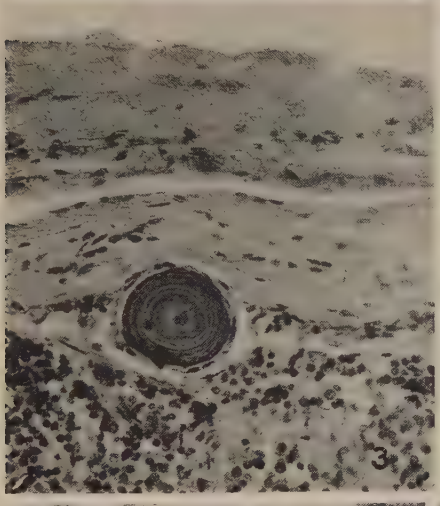
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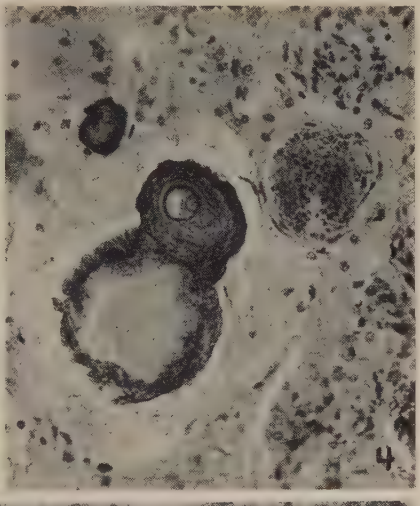
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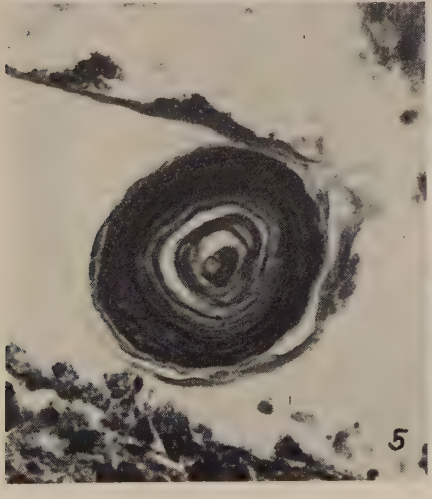
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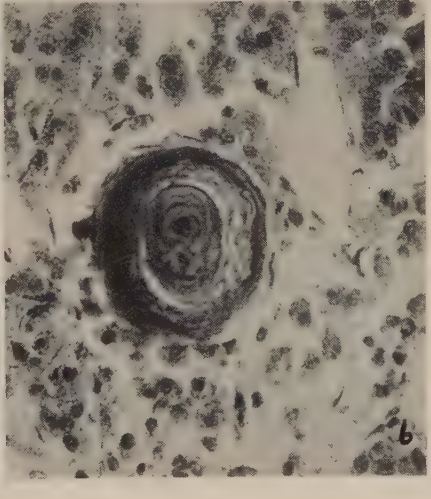
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PLATE 2

EXPLANATION OF FIGURES

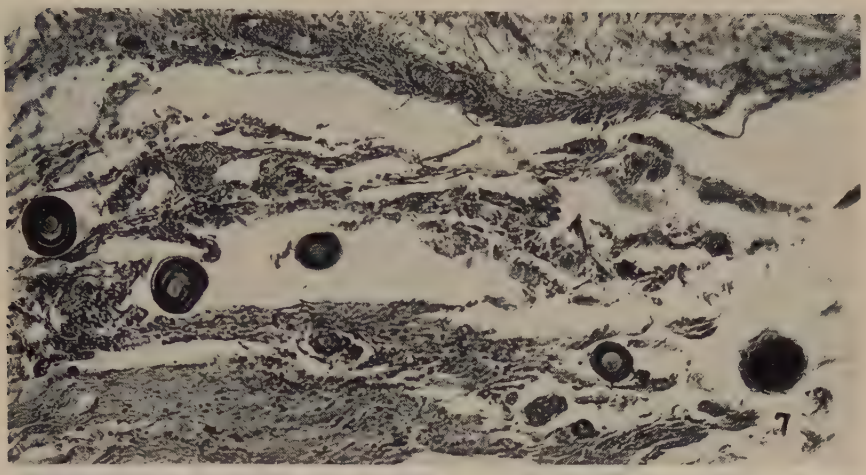
7 Multiple mesothelial concretions and mesothelium associated with capsule in region where stalk joins the infundibular process. × 117.

8 Mesothelial concretion with nearby mesothelium including a developing mesothelial concretion. These are located inside the pituitary between the stalk and the anterior lobe. × 234.

9 A degenerating and a normal double mesothelial concretion, with associated mesothelium, along outer edge of capsule of stalk. × 383.

10 Area of hyperplastic mesothelium in capsule near insertion of the stalk. × 720.

11 Area of hyperplastic mesothelium in capsule near insertion of stalk, including one enlarged mesothelial cell, an early stage in formation of a mesothelial concretion. × 720.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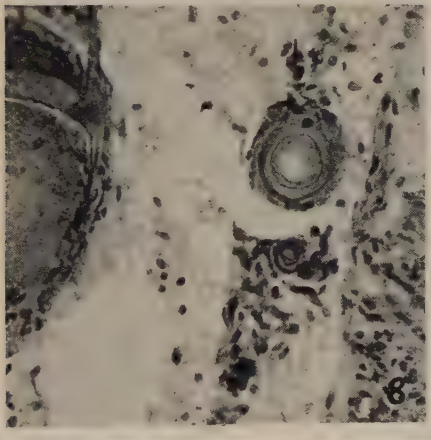
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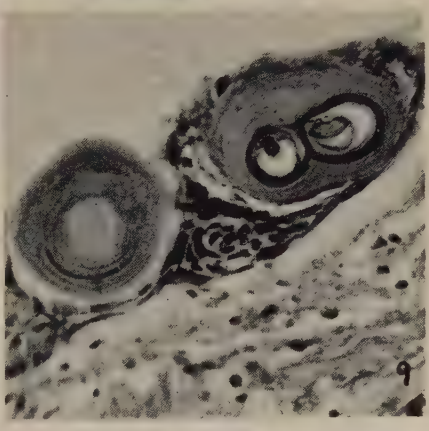
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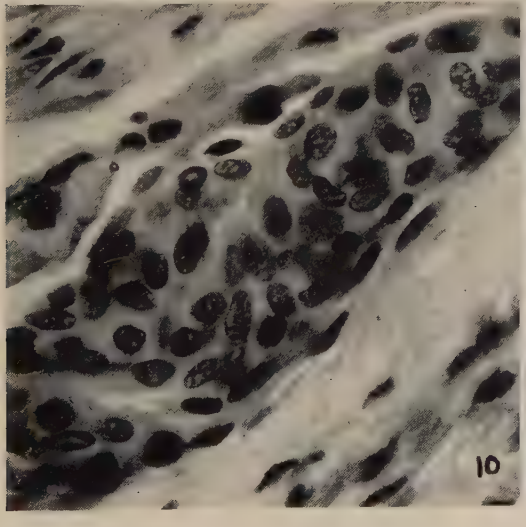
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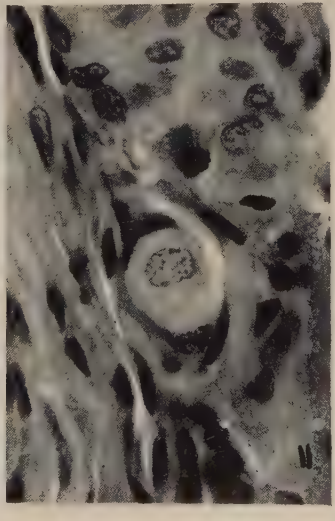
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PLATE 3

EXPLANATION OF FIGURES

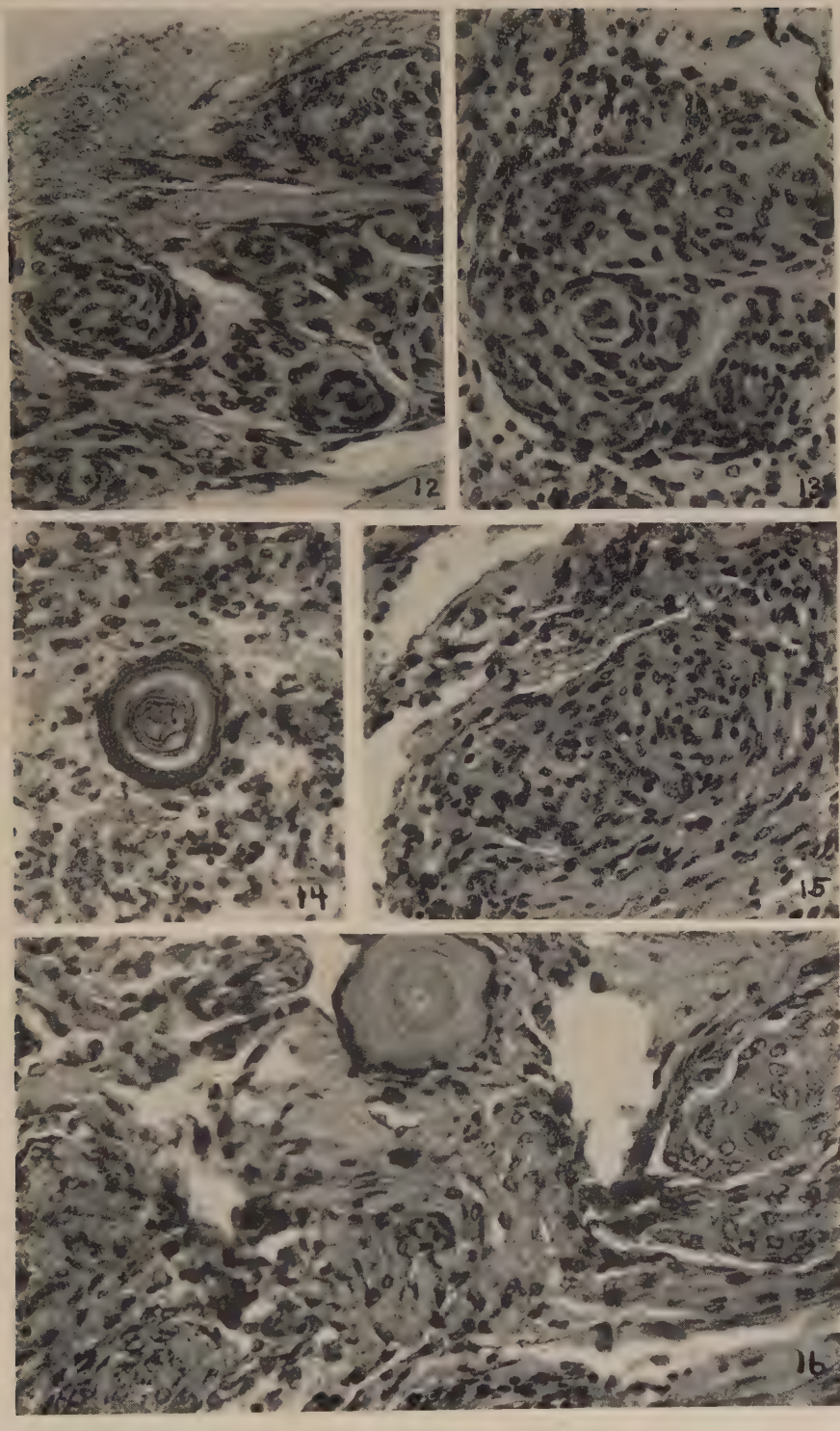
12 Area of mesothelium in capsule near insertion of stalk, showing whorl formation. × 383.

13 Area of mesothelium, with whorls, located among inflammatory cells that have largely replaced the infundibular process. × 383.

14 A mesothelial concretion surrounded by inflammatory cells in same specimen as preceding figure. × 383.

15 Mass of hyperplastic mesothelium in capsule near insertion of stalk into infundibular process. × 270.

16 Section through large area of hyperplastic mesothelium, including a mesothelial concretion, that has invaded a large part of the capsule near the insertion of the stalk. × 383.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THE RELATIONSHIP OF PARS TUBERALIS TO PARS DISTALIS IN THE HYPOPHYSIS OF THE RHESUS MONKEY

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TEN FIGURES

INTRODUCTION

In previous papers (Dawson, '37, '38) the regional restriction of both acidophiles and basophiles in the pars distalis of the hypophysis of the rabbit, cat and opossum was emphasized. In these animals the basophiles tended to be aggregated in, and the acidophiles excluded from, a median, antero-ventral region which merged dorsally into the basal portion of the pars tuberalis. On account of the morphological relationship this special region of the pars anterior was designated as the zona tuberalis of the distalis.

The existence of a zona tuberalis in mammals has not been widely recognized. The usual descriptions of the pars distalis imply that the several cell types are not arranged in any fixed pattern and that their distribution is a more or less random one. Von Soós ('34) however, clearly recognized that while the basophiles may be variously distributed within the anterior lobe of different mammals, they tended to occupy the same location in individual species. His descriptions are brief and his illustrations are inadequate as the drawings are based on median sagittal sections, usually the least favorable plane to demonstrate the distribution of basophiles and the relationships of the grouped basophiles to the other regions of the hypophysis. In the human gland the basophiles

are generally recorded as most numerous in the periphery with the acidophiles concentrated centrally but in the bovine hypophysis the distribution is reversed, basophiles predominating centrally with the acidophiles grouped peripherally (Smith and Smith, '23). In the rat basophiles are most numerous at the anterior and posterior margins of the gland (Severinghaus, '39).

Kirkman ('37) states that in the guinea pig the junctional zone of the pars anterior with the pars tuberalis is made up almost entirely of basophiles, both light and dark types, and that the basophiles are also most numerous in the middle of the anterior lobe, the acidophiles being much more abundant in the lateral extremities of the gland. Romeis ('40) in a review of the pituitary gland concludes that in man, cattle, pigs, cats and dogs acidophiles are most numerous in the lateral halves of the pars anterior while basophiles and chromophobes are grouped in a ventro-caudal and median zone. In man Floderus ('44) has described a restricted, antero-medial area of the pars anterior which lies at the base of the pars tuberalis and consists chiefly of basophiles and chromophobes.

In several whales (Jacobsen, '41; Hanström, '44) the pars tuberalis is continued, without interruption, deep into the pars distalis, thus, constituting a pars tuberalis, externa and interna. The interna consists of basophiles and tuberalis chief cells (chromophobes). In the tiger (Hanström, '46a) a median localization of basophiles and chromophobes, comparable to that of the domestic cat (Dawson, '37) is present, while acidophiles, along with scattered basophiles and chromophobes, predominate laterally. Hanström, however, concluded that localization of the basophiles and their relationship to the pars tuberalis in the tiger were too indefinite to permit classifying this region as either a pars tuberalis interna or a zona tuberalis; but in the Indian elephant, he described, within the pars anterior, a rather well-defined region, situated medially and anteriorly and continuously connected with the pars tuberalis which he designated as a

zona tuberalis. This region contained mostly basophilic and chromophilic cells, whereas acidophiles were predominant laterally and dorsally. In the shrew and hedgehog the distribution of the basophiles resembles, to a certain extent, the relation found in other mammals possessing a zona tuberalis, but Hanström ('46b) concluded that a true zona tuberalis was not present in these species.

A true zona tuberalis with dominating basophiles is not present in the fox (Hanström, '47), but in the polar and brown bears, there is a richly vascularized region in the anterior central part of the pars anterior which contains mostly chromophobes with some basophiles and a few acidophiles. On account of its relationship with the pars tuberalis Hanström ('47) feels that this region can justly be compared with the zona tuberalis of the rabbit (Dawson, '37) and pars tuberalis interna of the whales (Hanström, '44). Although the basophiles in the badger, weasel, mink and ferret are generally concentrated within the ventro-medial region of the pars anterior they are so mixed with scattered acidophiles that a definitive zona tuberalis could not be recognized but in the pole cat the restricted distribution of acidophiles is such that a zona tuberalis may be said to be present (Hanström, '47).

The regional subdivision of the pars anterior on the basis of restricted distribution of cell types is also evident in birds (Rahn, '39; Rahn and Painter, '41; Painter '42). Here the caudal margin of the base of the pars tuberalis marks the boundary between the so-called caudal and cephalic lobes, so that the cephalic lobe is at least analogous to, if not homologous with the zona tuberalis of the pars distalis of mammals.

From this review it is obvious that an antero-ventral and medial localization of basophiles in the pars distalis, in close relationship with the pars tuberalis, is not characteristic of all mammals. In some rodents the distribution of basophiles has no apparent relationship to the pars tuberalis while in many other mammals the relationship exists in varying degrees of intimacy, reaching its culmination in the whales were

a definite pars tuberalis interna is morphologically continuous with the external pars tuberalis. The local grouping or restricted distribution of cell types is in all likelihood a histological expression of a fundamental plan of organization of some physiological import. The manner of innervation is probably not the prime factor in determining this histological pattern, which is essentially a secretory pattern, since the nervous supply in most instances is too scanty to control secretory activity directly. It seems more likely that the vascular supply is of greater importance.

The cellular pattern or regional secretory response might be controlled, in part, by local changes in the blood supply but the major factor would seem to be the humoral products which occur in the median eminence following hypothalamic stimulation and are relayed to the pars distalis by way of the hypophyseal portal vessels. Morphological evidence in favor of the latter viewpoint has been summarized by Green ('46, '47a, '47b) and Green and Harris ('47) and significant physiological evidence of such a humoral link has been recently furnished by Markee, Sawyer and Hollinshead ('46) and Sawyer, Markee and Hollinshead ('47) in their studies of ovulation in the rabbit.

The purpose of this paper is to report the presence in the pars anterior of the rhesus monkey of a special region which seems comparable to the zona tuberalis of other mammals although the morphological relationships and distribution of cell types are not identical.

MATERIAL AND METHODS

These observations are based on the pituitary glands from 60 female monkeys and one thyroidectomized male. The glands were generously donated by F. L. Hisaw and C. G. Hartman from their experimental animals and have been accumulated during the past 10 years. All but 9 of the animals (females in poor health) had been subjected to a variety of treatments, either with single hormones or various combinations of hormones, with or without ovariectomy. For

present purposes it is sufficient to state that in many of these experimental animals, wide and sometimes very striking changes were produced in the cellular composition of the pituitary glands and that the changes in certain cell types followed a definite pattern which was related to regional differentiation within the anterior lobe.

The glands were fixed in either Bouin's or Helly's fluids or in a sublimate-saline-formol mixture (Dawson and Friedgood, '38). Complete serial sections 4 micra thick, of 55 entire glands and 6 lateral-halves were prepared. Most of the sections were cut in a plane parallel with the floor of the brain but sufficient sagittal series were prepared to give a more complete determination of the relationships of the several epithelial components of the gland. The horizontal sections, due to the manner of cellular distribution in the anterior lobe proved the more valuable.

The sections were stained with Heidenhain's azan modification of Mallory's triple connective tissue stain. With this method 4 tinctorially distinct cell types may be demonstrated. The more finely granulated, so-called ordinary acidophiles are stained yellow-orange; the large, coarsely granulated, carminophiles are deep red; the basophiles are varying intensities of blue, while the chromophobes are colorless or light pink. The additional tinctorial type, the carminophile, has been described in the anterior lobe of several other mammals as well as in other classes of vertebrates (Friedgood and Dawson, '37, rabbit; '40, cat; Oldham, '38, armadillo; Romeis, '40, man, cat, dog; Hartman and Wolfe, '46, dog; Hanström, '46a, tiger; 46b, European hedgehog; '47, badger, ferret). Apparently only Friedgood and Dawson have paid particular attention to the distribution of the carminophiles under varying physiological and experimental conditions (rabbit and cat). Moreover, it is still to be determined whether or not this similarity in staining capacity of the carminophile in unrelated species is evidence of similar functional capacity. In the rabbit and cat the carminophile has been interpreted as a special secretory phase of an acidophile or

potential acidophile. In the monkey, however, the Golgi apparatus of the carminophile both in form and position is very like that of the basophile. Hartman and Wolfe feel that a definite classification of this tinctorial cell type should be postponed since the absence of intergrading forms makes it impossible to regard it as a variant of either the usual acidophile or basophile. Nevertheless inability to assign this cell to a definite category or to determine its functional significance in all instances does not preclude using it as evidence of regional responsiveness within the anterior lobe.

This digression regarding the rather widespread occurrence of carminophiles in mammals is occasioned by the observation that many of the changes in the number and distribution of carminophiles in the pituitary gland of the monkey are centered in and about the zona tuberalis.

DESCRIPTION

General morphology

Before proceeding to a specific description of the zona tuberalis a brief account of the relationships on the component parts of the entire gland will be given. The neural lobe is solid and occupies its usual posterior position in this glandular complex. It is closely invested on its anterior surface by the pars intermedia. A residual lumen is commonly completely absent although it may be represented in occasional glands by one or several, narrow, elongated vesicles which frequently are ciliated. The pars intermedia is not uniformly developed in all glands. It varies greatly in thickness and always thins out posteriorly to disappear midway to the posterior midline of the neural lobe. On the anterior surface it may be greatly thinned or thickened locally, or even absent in some regions. The pars distalis is closely applied to the pars intermedia but usually is not extended posteriorly to enclose or partially clasp the fused pars intermedia-neural lobe.

Zona tuberalis of the pars distalis. The chief feature of interest in the general topography of the pars anterior is the

selective distribution of the several cell types which are so arranged that the pars anterior appears to be composed of two types of tissue, one occupying a median antero-ventral region, the other situated more laterally and posteriorly. Temporarily the medial region will be referred to as modified, the more lateral regions as unmodified, anterior-lobe tissue. This morphological pattern is most obvious in horizontal sections and is so marked that it may be recognized macroscopically. The rather definitely circumscribed antero-median area can be traced directly to the base of the pars tuberalis and consequently is regarded as constituting a zona tuberalis in the anterior lobe. In the monkey, however, the zona tuberalis is not a simple antero-ventral median region, as it is in most mammals possessing one, but it is divided during much of its extent along the anterior surface of the gland into two bilaterally symmetrical masses by a wedge-like extension of unmodified anterior-lobe tissue (fig. 9). In horizontal sections through this level the two areas appear roughly triangular in shape, with the bases of the triangles resting on the anterior surface of the pars distalis and the apices directed posteriorly and laterally far into the unmodified anterior-lobe tissue. Their medial and lateral boundaries are also delimited from the unmodified anterior-lobe tissue by relatively thick septa of connective tissue. This internal topography is usually reflected externally on the anterior surface of the anterior lobe. The bases of the triangular areas are usually marked by shallow grooves, separated by a low keel which represents the free margin of the wedge-shaped, unmodified pars anterior tissue which locally bisects the zona tuberalis tissue.

More ventrally the zona tuberalis is undivided and gradually becomes less distinctly limited from the rest of the anterior lobe tissue until at the ventral pole one tissue merges into the other. Dorsally the zona tuberalis also becomes single and median and as it approaches the base of the pars tuberalis the cells appear cytologically undifferentiated, i.e. in the region immediately adjoining the pars tuberalis and pars intermedia. This median antero-dorsal area appears to represent a zone of

transition and the cells gradually assume the cytological characteristics typical of cells of the definitive pars intermedia and pars tuberalis (fig. 8).

The solid infundibular stalk, which is bent sharply anteriorly as it emerges from the neural lobe, is partially embedded in the pars intermedia and pars tuberalis and is eventually invested by the pars tuberalis as it turns upward toward the base of the brain (Wislocki and King, '36, fig. 3).

Distribution of cell types in the pars distalis. Ordinarily the cellular population of the unmodified portion of the pars distalis is much more stable than that of the zona tuberalis. Usually the predominating cell type is the ordinary acidophile which stains with orange G. A variable but not conspicuous number of small chromophobes are also present. Although the basophiles tend to be aggregated in the zona tuberalis they are not confined entirely to this modified region but are also found rather diffusely and uniformly distributed among the ordinary acidophiles. These basophiles are usually relatively small and circular in outline and tend to occur singly. Their nuclei frequently lack a definite chromatin pattern and stain homogenously with anilin blue, obscuring to some extent the single nucleoli which react with azocarmine. The presence of these round, blue basophiles among the compactly grouped orange acidophiles gives a bird's-eye effect to the tissue. Under certain physiological and experimental conditions carminophiles may also be found in the unmodified regions of the pars distalis.

As indicated earlier, the zona tuberalis of the anterior lobe was originally distinguished in the rabbit and cat by the concentration of basophiles within it, to the almost complete exclusion of the acidophiles. No general statement can be made regarding the zona tuberalis in the monkey since great, almost kaleidoscopic, changes in the tinctorial types of cells localized in this region can be induced by various hormonal treatments or experimental conditions. These changes center primarily about the relative numbers and distribution of basophiles and carminophiles that are present.

Ordinary acidophiles also occur rather frequently in the more ventral portion of the zona tuberalis as well as in the lateral margins of the partitioned region, but in the medial borders of these bilateral areas, adjoining the wedge of unmodified tissue, the transition between zona tuberalis and ordinary anterior lobe tissue is rather abrupt (fig. 9). More dorsally the ordinary acidophiles are rather sharply restricted from the single, medial region which becomes morphologically continuous with the base of the pars tuberalis. In this paper no attempt will be made to discuss the many types of change that may be induced in the cellular population of the zona tuberalis since a consideration of several factors which have been found to influence the occurrence, number, distribution and behavior of carminophiles will be the subject of a later report. Only a few examples, sufficient to indicate the responsiveness of this region, will be cited.

Following ovariectomy the entire zona tuberalis is occupied almost exclusively by large basophiles and the effect of castration can also be observed in the scattered basophiles of the unmodified portion of the pars anterior. After thyroidectomy a somewhat comparable picture is observed but the basophiles outside the zona tuberalis show little change. In castrated females treated for long periods with estrogen alone, or estrogen and progesterone with or without pregnancy urine hormone, the carminophiles become the conspicuous cell type. They may occur in scattered irregular clusters of varying size, or be massed almost solidly throughout the entire zona tuberalis (fig. 10). In more extreme cases the carminophiles are not confined entirely to these special areas but may be widely scattered through the unmodified portion of the pars anterior, showing in some instances a secondary tendency to be concentrated in a narrow band paralleling the pars intermedia. Thus, in addition to the gross morphological pattern within the pars anterior due to the regional differentiation of the zona tuberalis there is also a physiological pattern of response. This latter pattern is made evident by relatively orderly progressive or retrogressive changes in the relative

numbers of chromophiles, especially basophiles and carminophiles, present.

Vascular supply. The vascular supply of the hypophysis of the rhesus monkey has been carefully described by Wislocki and King ('36) and Wislocki ('38). The blood supply of the neural process is essentially independent of that of the anterior lobe. The anterior lobe is supplied by branches of the superior hypophyseal arteries and by portal venules. Offshoots of the arterial vessels go either to the stalk or to the neck of the gland. Those entering the neck penetrate the anterior lobe and terminate in characteristic sinusoids. No systemic veins accompany the arterial supply. The portal venules consist of a number of small, thin-walled vessels embedded in the pars tuberalis on both sides. These connect the plexiform capillary bed of the infundibular stalk, with the sinusoids of the anterior lobe. The vascular plexus of the infundibular stalk, in turn, is connected with the fine capillary bed of the hypothalamus proper by anastomoses which occur at the border of the median eminence. The efferent drainage of the anterior lobe is by numerous short lateral hypophyseal veins which emerge from the lateral poles of the gland to enter the cavernous sinuses.

While Wislocki ('38) does not emphasize the bilateral distribution of the portal venules within the body of the anterior lobe, some of his illustrations (especially fig. 4) of frontal sections of injected specimens give evidence of such a distribution.

Wislocki's figures only include sagittal and frontal sections. The distribution of the venules would probably have been more obvious if he had also prepared horizontal sections, such as were used so successfully in this study to determine the extent of the zona tuberalis within the pars distalis. There seems to be no doubt but that the zona tuberalis is supplied primarily by the portal venules. These large irregular vessels can be readily recognized in the zona tuberalis and present a striking contrast to the narrower characteristic sinusoids uniformly present in the unmodified anterior lobe tissue. The

sources of the lateral hypophyseal veins can be first recognized histologically in the coarser connective tissue trabeculae associated with the apices of the bilateral extensions of the zona tuberalis which reach postero-laterally far into the lateral halves of the pars anterior.

DISCUSSION

There is increasing evidence in support of the hypothesis that the secretory activity of the anterior lobe is not under direct neural control by the hypothalamus but that the hypothalamic influence is exerted by way of a humoral link or relay through the hypophyseal portal vessels. Our observations on the frequent localization of the secretory response, especially of basophiles and carminophiles in the zona tuberalis gives indirect support to such conclusion. Indeed one is tempted as a result of a study of the manner of spread of the pattern of granulation and degranulation throughout the entire pars anterior to postulate further that in some instances the portal flow might be shunted through the region of the zona tuberalis almost directly to the lateral hypophyseal veins with little portal blood entering the sinusoids outside this restricted region. Such a diversion of portal blood would not necessarily deprive the tissue beyond the zona tuberalis of an adequate blood supply since the sinusoids are fed by both portal venules and terminals of the superior hypophyseal arteries. This speculation implies that the activity of the anterior lobe may be further modified through local or regional control of the intrinsic blood supply—a suggestion in keeping with the rather general observation that many of the nerve fibers in the pars distalis are of autonomic origin (Drager, '47; Green, '46; Green and Harris, '47).

It should be noted that Brooks and Bard ('42) found that after practically complete section of the hypophyseal stalk, mature female monkeys failed to menstruate for a period of several months, after which regular cycles, from 36 to 42

days in duration, were reestablished. No definite conclusions can be drawn from these observations since the rates of regeneration of portal vessels and nerve fibers were not determined.

A carminophile response in the anterior lobe, which follows coitus, has been demonstrated in the rabbit and cat, two animals in which a zona tuberalis has been described (Dawson, '37). In the cat a definite pattern of response was observed (Dawson, '46; Friedgood and Dawson, '40) but in this instance the carminophiles first appeared in two lateral zones far from the region of the zona tuberalis. In the rabbit the carminophile reaction occurred in irregular patches throughout the unmodified anterior lobe tissue without any apparent topographical relation to the zona tuberalis (Friedgood and Dawson, '38). It is not possible to explain such variations in the secretory response, but these meager observations suggest that localization of changes in granulation of the chromophiles is the result of an interplay of several factors—some of which are extrinsic, other intrinsic. Much of this discussion is based on the assumption that the carminophile reaction has a specific physiological significance which seems probable. Whether or not it has the same significance in quite unrelated species is still to be determined.

SUMMARY

A median antero-ventral region in the anterior lobe of the pituitary gland of the monkey, characterized primarily by the absence, or restricted distribution, of ordinary acidophiles (stained by orange G with the azan method), is described. On account of the topographical relationships with the pars tuberalis it is referred to as a zona tuberalis. This region is single ventrally and dorsally but is divided into two bilateral areas for a short distance on the anterior surface of the gland. The hypophyseal portal venules appear to be distributed primarily within the zona tuberalis.

The cellular population of this delimited area within the pars anterior varies strikingly under different physiological

and experimental conditions. The chief changes relate to the relative numbers of basophiles and carminophiles (stained with azocarmine) which are present. The remaining portion of the pars anterior appears to be much more stable and does not ordinarily show such marked fluctuations in the relative proportions of basophiles, ordinary acidophiles and carminophiles.

The regional responsiveness observed in the anterior lobe is attributed to the distribution of the portal venules which are believed to constitute a humoral link between the hypothalamus and anterior hypophysis.

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PLATE 1

EXPLANATION OF FIGURES

All figures of this plate were outlined at the same magnification (× 8) and are based on complete serial sections cut 4μ thick and stained by Heidenhain's azan method. The 8 diagrams illustrate the relationships of the several parts of the hypophysis: unmodified pars anterior, clear; zona tuberalis of pars anterior, solid dots; pars intermedia, solid black; pars tuberalis, open circles; infundibular process, irregular lines.

1 Mid-sagittal section of the pituitary gland. Dorsally there is a gradual transition from typical zona tuberalis to pars tuberalis. The local medial division of the zona tuberalis into two lateral masses (fig. 4) makes this tissue appear interrupted in a median sagittal section. The numbered lines (2 to 8) show the approximate levels at which horizontal sections of another gland were drawn in figures 2 to 8.

2 to 8 Horizontal sections drawn from successively higher levels of the gland to show chiefly the extent and topographical relationships of the special region of the pars anterior designated as the zona tuberalis. The histological continuity of three components, zona tuberalis, pars tuberalis and pars intermedia is indicated in figure 8. The transition from one type of tissue to another is not abrupt but is made from a common region in which the cells show none of the specific characteristics of any of the three tissues.

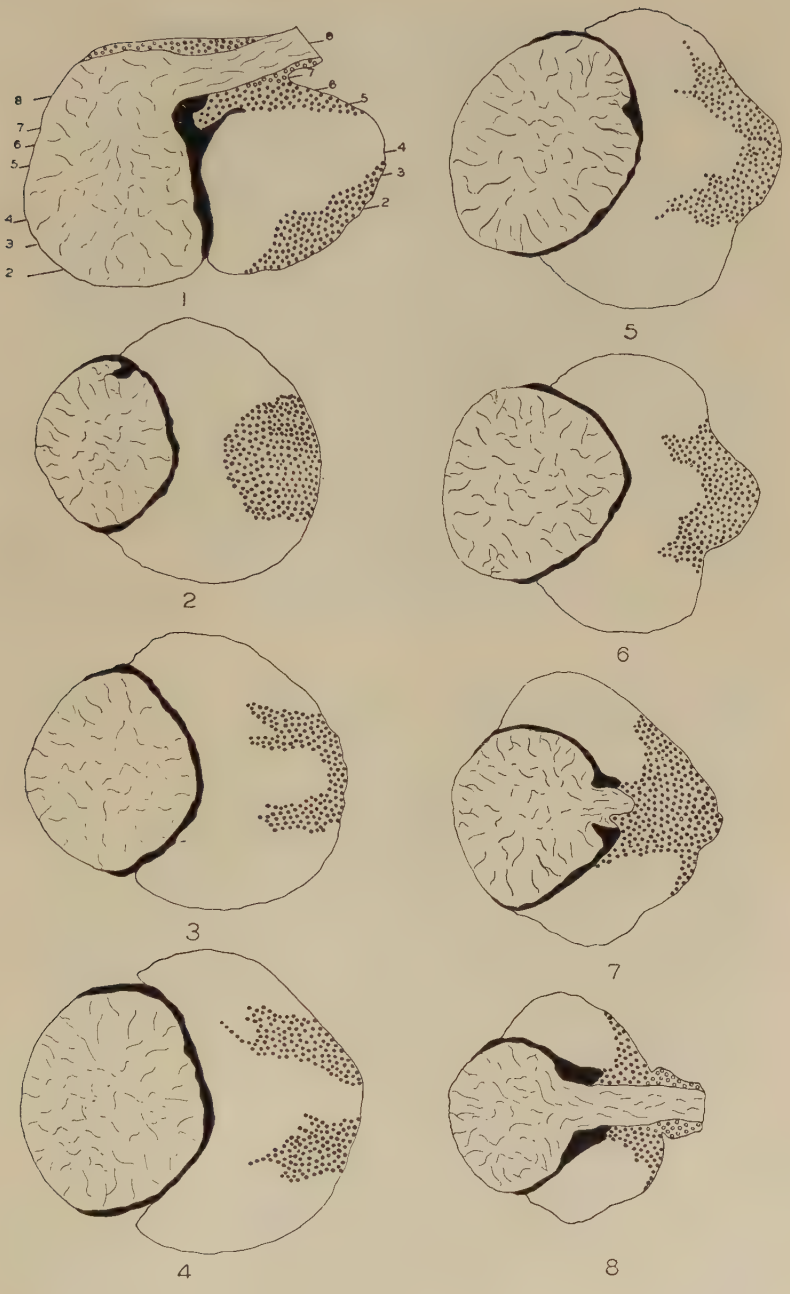
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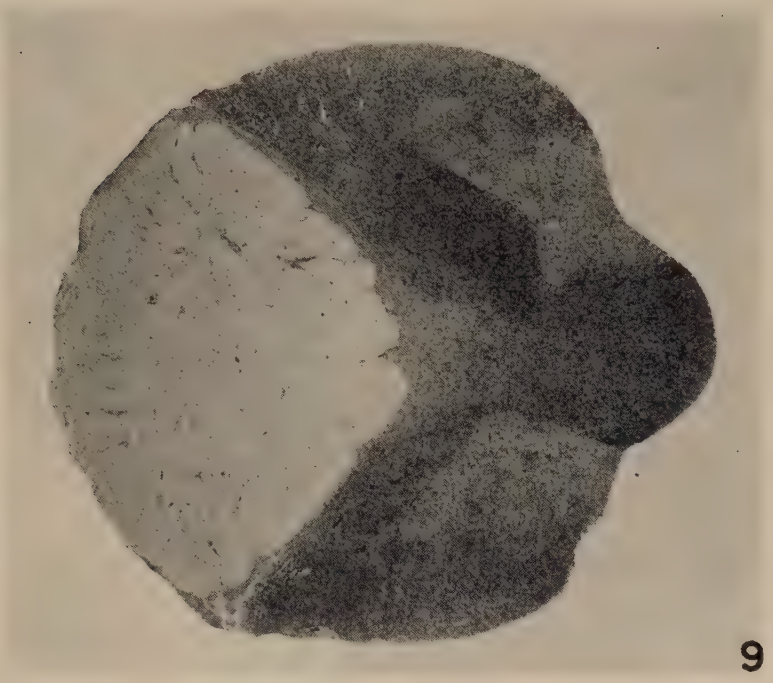
PLATE 2

EXPLANATION OF FIGURES

9 A photomicrograph (× 18) of a horizontal section of the pituitary gland at approximately the same level as that of figure 4, showing the regional differentiation within the anterior lobe. The zona tuberalis (at this level) appears as two bilateral areas, consisting chiefly of chromophobes and basophiles, with few scattered carminophiles. In this monkey, the follicles were grown with follicle-stimulating hormone and luteinizied by intravenous injections of pregnancy urine hormone. The luteal tissue was maintained by continuing subcutaneous injections of chorionic gonadotropin for 16 days. The animal was autopsied on the fourth day of uterine bleeding which had begun on the thirty-fourth day of the experiment.

10 A photomicrograph (× 20) of a horizontal section of a pituitary gland at a level comparable to that of figure 9. The apparent asymmetry is due to faulty orientations in sectioning. In this gland the carminophiles are numerous and concentrated chiefly in the bilateral areas of the zona tuberalis. This monkey had received the following treatments before the removal of the hypophysis: 28 days after bilateral ovariectomy, 1000 i. u. estrogen daily for 20 days; then 1000 i. u. estrogen plus 2 mg. progesterone daily for 11 days; followed by 1000 i. u. units estrogen plus 2 mg. progesterone plus 1 mg. pregnancy urine hormone for 13 days.



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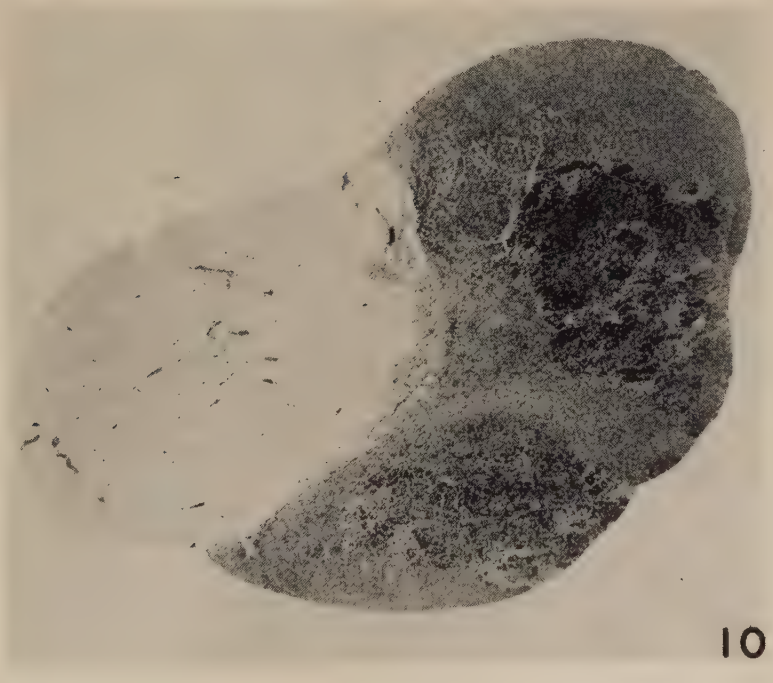

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THE ANATOMY AND CYTOLOGY OF THE PITUITARY GLAND OF THE GOLDEN HAMSTER (CRICETUS AURATUS)

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SEVENTEEN FIGURES

Since recent literature shows evidence of the increasing use of the golden hamster (Cricetus auratus) in laboratory investigations, studies of the normal anatomy and histology of the animal should prove useful as a basis for future researches. Those descriptions of the normal histology of the pituitary gland of the golden hamster which have appeared in the literature (Vasquez-Lopez, '44 and Koneff, Simpson and Evans, '46) are isolated and their presentation secondary to the main purpose of the investigation. Hence, it seemed that a descriptive study of the normal histology of the pituitary gland of the golden hamster would have practical value as a preliminary to further studies of hypophyseal activity in this animal.

Further, since the changes induced in pregnancy and by castration in the various laboratory forms have been widely referred to in endocrinological investigations, it appeared that a preliminary study of the effects of these conditions on the pituitary of the golden hamster might also be of value.

This presentation, therefore will confine itself to the normal histology and cytology of the pituitary gland of Cricetus auratus, and a preliminary study of changes which occur during pregnancy and following castration in the male.

MATERIALS AND METHODS

The animals used in the present study were members of a small colony of golden hamsters maintained on a diet of mixed grain supplemented by dog chow pellets and fresh greens provided daily. The pituitaries of a total of 56 animals were studied.

For histological study of the normal state the pituitaries of 19 males and 22 females were used. The males ranged in age from two to 10 months and the females from three to 9 months. The average weight of the males was 88 gm and of the females 92 gm. These served as controls for the two experimental groups in which castration and pregnancy effects were studied. All 7 males were castrated at the age of two to two and one half months. Study of the pituitary was made after an 84 day period of castration in three of the animals, after a 110 day period in one animal, and after a 133 day period in the remaining three. At the time of sacrifice the average weight of the first group had increased 19 gm, the weight of the single animal 20 gm, while that of the latter group had increased 53 gm. The average weight of the 8 pregnant animals at the time of sacrifice was 102 gm and the range in age from two and two thirds to four and two thirds months.

The animals were killed by carbon monoxide asphyxiation and immediately decapitated, the brain and most of the skull removed, and the pituitary fixed in situ. Final dissection of the gland followed fixation. Helly's and Dawson-Friedgood's formalin-sublimate were used as fixing agents for most of the glands. The Helly-fixed tissues were stained in haemalum and triosin for the study of general anatomical features and in the Masson stain for cellular differentiation. Dawson-Friedgood's azocarmine technique following the formalin-sublimate fixation was employed as an additional differential stain. For verification of cytological detail 7 pituitaries, including at least one of each of the aforementioned groups were

fixed and stained by the Severinghaus technique. After fixation the glands were cut in the median longitudinal plane into two equal halves; one half served for Golgi demonstration, while the other was treated to show mitochondria.

All glands were sectioned in paraffin at three to 5 micra. Most of the study was made on sagittal and transverse serial sections, although a frontal series was included for additional topographical study.

Normal glands fixed in Helly fluid and stained by the Masson technique were used for differential cell counts. These were cut serially in the transverse plane at 5 micra. Random observations made on several glands and a preliminary comparative count of various regions of single sections through the entire series of one gland showed no significant difference in the number of cells present. Therefore, to obtain a regular sampling through the entire gland, a specific area in the antero-ventral region of the anterior lobe was chosen and cells falling within this area on every twentieth section of the gland were counted. An ocular counting chamber was used; each section was oriented to the same position under low power, the oil immersion objective was then switched into position, and all cells which were enclosed within the square of the counting chamber were counted. In this way an average of 1290 cells per gland were counted.

Since the purpose of the counts was simply to verify superficial observations concerning relative percentages of cells present in both sexes of the animal, but a few glands were counted. This method does not demonstrate absolute numbers of cells present in the gland nor their regional distribution; it merely indicates relative percentages of cells present. There is no attempt made to relate physiological activity to morphological changes in the gland and therefore the method used seemed adequate. The figures obtained are subjected to a brief statistical analysis (Burn, '37) which is included in table 1.

OBSERVATIONS

General anatomy and cytology

The pituitary of the golden hamster consists of a pars anterior, pars intermedia, pars nervosa and a pars tuberalis. The gland is somewhat flattened dorso-ventrally and is broadest at the anterior end (fig. 12). The pars nervosa is closely

TABLE 1

Differential cell counts on the pars anterior of the pituitary of the golden hamster

CELL TYPE	MEAN	STANDARD ERROR OF THE MEAN	SIGNIFICANT DIFFERENCE (COMPARED TO THE MALE)
	Females		
Acidophil	27.1	3.4	1.9
Basophil	18.1	1.1	2.0
Chromophobe	54.8	3.0	0.5
	Males		
Acidophil	20.8	0.6	
Basophil	22.5	1.9	
Chromophobe	56.7	2.1	

This statistical analysis is based upon the following results.

ANIMAL	TOTAL NO. OF CELLS COUNTED	PER CENT ACIDOPHILS	PER CENT BASOPHILS	PER CENT CHROMOPHOBES
Estrus female (B-20)	1283	26.6	20.4	53.0
Estrus female (B-18)	1493	20.8	19.7	59.5
Estrus female (BB-10)	1560	30.8	21.0	48.2
Estrus female (B-5)	1389	25.2	15.6	59.2
Metestrous female (B-15)	1415	18.2	18.0	63.8
Metestrous female (R-5)	972	41.2	13.9	44.9
Male (B-2)	926	21.3	28.6	50.1
Male BB-12	1427	21.9	26.3	51.8
Male BB-13	1491	19.7	22.2	58.1
Male B-6	1301	22.3	21.4	56.3
Male B-7	1179	19.2	20.8	60.0
Male H-1	1040	20.5	15.5	64.0

applied to the pars intermedia, the two lying dorsally and anteriorly, separated from the pars anterior by a slight cleft. The pars tuberalis extends along the infundibular stalk forming a cap about it. A cord of cells connects the pars anterior to the pars intermedia at both the anterior and posterior regions (figs. 1, 8).

The pars anterior occupies the greater portion of the gland. In a cross section of the anterior end, the broad lateral portions of the lobe are seen to narrow markedly toward the midline where they form a thin region inferior to the pars intermedia and pars nervosa (figs. 2, 7). This region gradually thickens posteriorly, until, near the caudal end, the anterior lobe extends beyond the overlying intermedia and nervosa and is of uniform thickness in cross sectional view. The pars intermedia surrounds the pars nervosa laterally and ventrally and is separated from the latter by a well demarcated line which is occasionally broken by cords of epithelial cells which extend into the nervosa tissue. The most dorsal portion of the pituitary body consists of an oval shaped pars nervosa, which is continuous with the infundibular stalk (figs. 3, 10).

Pars anterior. The cells of the pars anterior are arranged in irregular anastomosing cords separated by capillary channels. In cross section, these are seen as nests made up of varying numbers of cells, most frequently 8 or 9. Acidophils, basophils, and chromophobes may be present in a single cord or nest (fig. 11). Glands of both sexes show some larger basophiles bordering on the capillaries. These cells, as well as the acidophils which lie among them at the capillary wall, often appear considerably elongated. In both sexes acidophils and small basophils lie along the cleft which separates the anterior lobe from the intermediate lobe. The percentage of acidophils is slightly higher in the female, whereas the male shows a slightly higher percentage of basophils (table 1).

The cells in the two sexes present essentially the same cytological detail. The basophils, which are larger than the acidophils contain both coarse and fine granules tending to clump about the periphery of the cell. Lightly and deeply staining basophils can be observed in both sexes, although the heavier staining densely granulated basophil is more plentiful in the male while the pale cell is more frequent in the female (fig. 11). The nucleus of the basophil may vary from a densely chromatic appearance to a more vesicular type, in which the chromatic material is aggregated at the periph-

ery. The Golgi apparatus in the basophil lies to one side of the nucleus. It is semicircular in outline and consists of a fairly dense network of threads within which the cytoplasm appears vacuolated (figs. 5, 16). Mitochondria are present throughout the cytoplasm of the cell but appear most concentrated in the region of less granular cytoplasm immediately surrounding the nucleus. Frequently mitochondria are massed to one side of the nucleus near the region of the Golgi material (figs. 6, 17).

In the larger acidophils the cytoplasmic granulation is more concentrated to the side of the nucleus, whereas in the smaller rounded acidophils there is an even distribution of cytoplasmic granulation (fig. 11). The acidophil contains a faintly staining vesicular nucleus which shows a peripheral clumping of chromatic material. The Golgi network closely caps the nucleus in the larger triangular shaped acidophils and may continue around the nucleus. However, the latter position of the Golgi material is more characteristic of the small rounded acidophil in which a capping by the Golgi network at one side of the nucleus is much less marked and sometimes absent (figs. 4, 16). The mitochondria, which are sparse in the acidophils are more scattered than in the basophils (figs. 6, 17).

The chromophobes are most easily distinguished by their prominent nuclei set in a faintly stained or completely chromophobic cytoplasm. The cell outlines are not well defined (figs. 7, 8).

The cells of the richly vascular pars anterior are set in a collagenous reticulum containing within nests of acidophils and chromophobes a brightly stained hyaline substance which at times appears laminated. In addition, occasional colloidal vacuoles are present which seem to be associated with the basophils. These vacuoles are more prevalent in the pituitaries of the older males. In these, also, are present numerous chromophobic spaces giving a coarse network appearance to the stroma of the anterior lobe. In the pituitary body of one of the 9 to 10 months old males, the network is so extensive that the entire peripheral region of the anterior lobe is prac-

tically devoid of cells (figs. 3, 10). Many of the cells appear to have been washed out and at the edges of the clear areas one can find cells apparently undergoing disintegration. That part of the cell which borders the open space has no clear outline, and the entire cell is markedly reduced in size.

In the above-mentioned male gland and in another of the same sex and age a cyst is present in the antero-ventral region of the anterior lobe. In each gland the cyst, which is lined with low cuboidal ciliated epithelial cells, is seen to contain brilliantly stained hyaline substance which is massed within the central area.

Pars intermedia. This lobe, as has been previously pointed out, is separated from the pars anterior by a cleft and lies in close proximity to the pars nervosa (fig. 9). Cords of epithelial cells at the anterior and posterior margins of the pars intermedia extend into the pars nervosa making the line of demarcation between the two more irregular in these regions. The most prominent feature of this portion of the hypophysis is the vesicular, ovoid nuclei. All of the intermedia cells are faintly basophilic in their staining reaction. They are closely packed, show rather indistinct outlines and sparse fine cytoplasmic granulation (fig. 9). The Golgi material occupies an asymmetrical position typical of the basophil. There is an underlying reticulum, a limited number of blood vessels, and occasional small hyaline masses are seen in the lobe.

Pars nervosa. The greater part of the tissue of the pars nervosa consists of a mass of neuroglia, ependyma cells, and closely interwoven fibers. Occasional epithelial cells which resemble those of the intermedia are present. Small hyaline masses are visible, scattered throughout the lobe. Numerous blood capillaries and sinuses are present (figs. 8, 9).

EXPERIMENTAL MODIFICATIONS

Pregnancy changes. The pituitary of the pregnant female shows many basophils in varying stages of degranulation. Some of the glands removed within the last two days prior to parturition show numerous sharply outlined deeply stained

granular acidophils and lesser numbers of large clearly defined basophils which lie along the blood capillaries. These cells do not show elongation at the capillary wall as do those of the normal animals as previously noted (fig. 15). No clear difference in the appearance of the Golgi from that of the normal was noted.

In one of the pituitaries obtained from a pregnant animal two cysts are present, of the same type previously described. One of them, completely enclosed within the pars anterior, contains a deeply basophilic hyaline mass and is lined with ciliated cuboidal epithelium. The other is a large structure which lies in the dorsal region of the gland and extends into all three lobes. Lateral sections of the pars anterior show the cyst as containing rather large masses of desquamated, irregular shaped basophilic cells. These are located at the periphery of the cyst and gradually disappear leaving only the hyaline mass within the lumen.

Castration changes. There is an increase in basophils in the male castrate pituitary and the heavily granulated deeply staining type becomes more prevalent. There is some increase in the number of vacuolated basophils in the glands of the castrated males, which becomes more marked in the older castrates (fig. 13). The vacuolation varies from a condition in which numerous tiny vacuoles are present in the cytoplasm to one in which cells show one or two large vacuoles which may occupy a half or more of the total area of the cell. The nuclei of many of the basophils, including both the vacuolated and non-vacuolated cells, quite consistently take a more basophilic stain and become more irregular in outline. The negative image of the Golgi is seen rather distinctly in many of the cells, although again, no marked difference from the normal occurs in the osmicated sections.

DISCUSSION

In its general anatomical features the pituitary body of Cricetus auratus resembles that of various other mammalian laboratory forms. In the positional relationships of the three

lobes, namely, the pars anterior, the pars intermedia and the pars nervosa, the pituitary body most closely resembles that of the rat. One outstanding difference is the unusually long caudal extension of the anterior lobe in the hamster. The pars nervosa is a solid mass of tissue resembling the nervosa tissue of the guinea pig hypophysis (Vanderburgh, '17). It differs from such forms as the cat in that the infundibular cavity does not extend into the tissue of the pars nervosa (De Beer, '26). The gland has a fairly prominent intermedia which has already been described by Vazquez-Lopez ('44).

The observations made in the present report on the histology of this portion of the normal gland are in accord with those of Vazquez-Lopez ('44). Koneff, Simpson and Evans ('46) describe an hypertrophy of the pars intermedia and an invasion of the neural and anterior lobes following stilbestrol treatment, as had Vazquez-Lopez ('44) following estrogen treatment. The observation of Kirkman ('37), working with the guinea pig, bears some resemblance to these findings. He noted an increase in colloid in the pituitary during the latter stages of pregnancy, and a possible migration of cells from the pars intermedia into the pars nervosa, at parturition. Thus, a more thorough study of cyclic changes in the pituitary of the normal female and an analysis of the entire gestation period in the hamster with special reference to intermedia changes may prove of value.

The size relationships of the cells of the anterior lobe confirm those reported by Koneff, Simpson and Evans ('46). Severinghaus ('33) has noted in the rat an angularly outlined acidophil as well as the rounded or ovoid acidophil, which is in accord with the observation of these cells in the hamster anterior pituitary. Although the specific percentages of cell types differ from those found for the hamster, a similar greater acidophilia in the female relative to the male was noted in the human (Rasmussen '29, '33), and in the guinea pig (Kirkman, '37). However, these authors noted a considerably greater percentage of acidophils over basophils in both sexes as compared to figures obtained for the hamster in the present

report. Thus, although it seems evident that the sexual dimorphism noted is a real one, it is quite possible that counts on a larger number of animals which would include also a careful analysis of all regions of the gland might give somewhat lower percentages of basophils in both the male and female glands, and upon analysis a higher significant difference particularly with respect to numbers of acidophils in the two sexes.

The appearance of the Golgi apparatus in both acidophils and basophils is similar to that which is described by Koneff, Simpson and Evans ('46) in the hamster and in other common laboratory forms by other investigators: Severinghaus ('33) in the rat; Kirkman ('37) in the guinea pig; and many other investigators. The mitochondrial distribution similarly is comparable to that noted for other forms.

The appearance of vacuolation in the normal anterior lobe of the golden hamster has been noted by Koneff, Simpson and Evans ('46). These authors identify the vacuolated cells as modified acidophils. In the present study it has not been possible to verify this observation. Some vacuoles which remain unstained are visible in most of the glands; however, extensive vacuolation and the network of unstained open spaces which the above authors have described are visible in the pituitaries of the older males especially. In addition, basophilic colloid vacuoles are observed in the basophils of the normal males and these seem to show a progressive increase with the age of the animal. In the glands of both sexes one observes also small masses of basophilic hyaline material which usually lie surrounded by acidophils. No correlation of these three types of vacuolation can be made on the basis of this investigation. Although the presence of hyaline or colloid material and some vacuolation in cells has been noted in other forms, none seem to show the unusual degree of vacuolation found in the anterior lobe of the normal hypophysis of the hamster.

The frequency of the cysts noted in some of the pituitaries of this series seem to approach the conditions found in the domestic pig (Maurer and Lewis, '22), the dog (de Beer, '26),

the human (Lewis and Lee '27; Rasmussen '28), the rabbit (Dawson,'37) and the guinea pig (Kirkman, '37). However, a greater number of animals should be examined for further confirmations.

The increase in degranulated basophils and appearance of increasing numbers of acidophils noted in glands obtained from the pregnant animals is essentially similar to that described in other animals. Since the animals studied were all killed during the latter half of the gestation period, it has not been possible to trace the changes occurring in each of the cell types of the anterior pituitary during the entire period of pregnancy, in the present report. The observations made, thus, agree most specifically with those made on the rat (Wolfe and Cleveland, '33) and the guinea pig (Kirkman, '37) for the latter period of pregnancy.

Castration in the male hamster produces an increase in the basophilia of the anterior lobe corresponding to the general picture which has been established for other mammals. The typical signet ring castration cell, as has been described in the rat by Addison ('17) and verified by later investigators, does not appear to be a marked feature of the hamster castrate pituitary. With reference to these findings in the hamster, certain studies of the castration changes noted in other forms may be cited. Kirkman ('37) describes an increase in colloid and the absence of the signet ring castration cell in the guinea pig pituitary following castration. Smith, Severinghaus, and Leonard ('33) report no such cell present in the rabbit. Biggart ('34), in a study of the human castrate pituitary, reports the appearance of vacuolation in a large type of chromophobe, in which he notes some basophilic granulation, but again, the typical signet ring type of cell is not described. It is interesting to note, also, that Severinghaus ('37) cites an increase in basophilic vacuolation in senile human females, although no signet ring types of cells are found in either these or in aged males. Further study of the vacuolation in the normal gland with an analysis of the correlation between time of appearance and amount will be val-

uable for comparison to the experimental castrate picture and for investigation of cytological changes produced by physiological castration, or senility, in this form. Such studies are now in progress.

In the present report, the resemblance of Cricetus auratus to other mammals in the general anatomy and cytology of its pituitary gland has been indicated. Certain variations appear, notably the fairly extensive network of chromophobic areas present in many normal glands, and the prominence of the pars intermedia.

SUMMARY

1. The pituitary gland of Cricetus auratus is composed of 4 distinct lobes, the ventral pars anterior, the pars intermedia, the dorsal pars nervosa and a pars tuberalis which extends upward along the infundibular stalk from the pars anterior.

2. The pars anterior, which is the largest portion of the gland contains three distinct cell types, the basophil, the eosinophil and the chromophobe.

3. The female gland shows a slightly greater acidophilia, whereas the male shows a somewhat greater basophilia.

4. The nature and distribution of Golgi material and mitochondria is similar to that described for other mammals.

5. The pars intermedia is composed of several layers of light basophilic cells which contain conspicuous nuclei.

6. The pars nervosa is a well vascularized mass of neural tissue in which scattered epithelial cells are seen. No infundibular cavity is present in this lobe.

7. During the latter period of pregnancy, the pars anterior contains an increased number of degranulated basophils and an increasing number of acidophils appearing very late in the period.

8. The anterior lobe of the castrate male is characterized by an increased basophilia and some increase in basophilic vacuolation.

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PLATE 1

EXPLANATION OF FIGURES

All figures are camera lucida drawings. Figures 1, 2 and 3 show the relationships of the various parts of the gland: pars anterior, coarsely stippled; pars intermedia, large solid dots; pars nervosa and infundibular stalk, finely stippled; pars tuberalis, solid black.

1 Median sagittal section of the pituitary gland of a 3⅓ month-old female. This is a drawing of the section shown in figure 8. × 38.

2 Cross-section through the mid-region of the gland of a 4⅓ month-old female. This is a drawing of the section shown in figure 7. × 38.

3 Frontal section through the mid-dorsal region of the gland of a 9 to 10 month-old male. Extensive vacuolation of the pars anterior may be seen, also. This is a drawing of the section shown in figure 10. × 38.

4 Individual acidophil of the pars anterior of a 4⅔ month-old female showing the Golgi material capping the nucleus. × 1100.

5 Individual basophil of the pars anterior of a 6⅔ month-old male showing the Golgi material displaced to one side of the nucleus. × 1100.

6 Individual cells of the pars anterior of the hamster in figure 5 showing mitochondria. Note the distribution of the mitochondria around the nucleus and in the region of the Golgi network in the single large basophil. Fewer mitochondria and a more scattered distribution of them can be seen in the two smaller acidophils. × 1500.

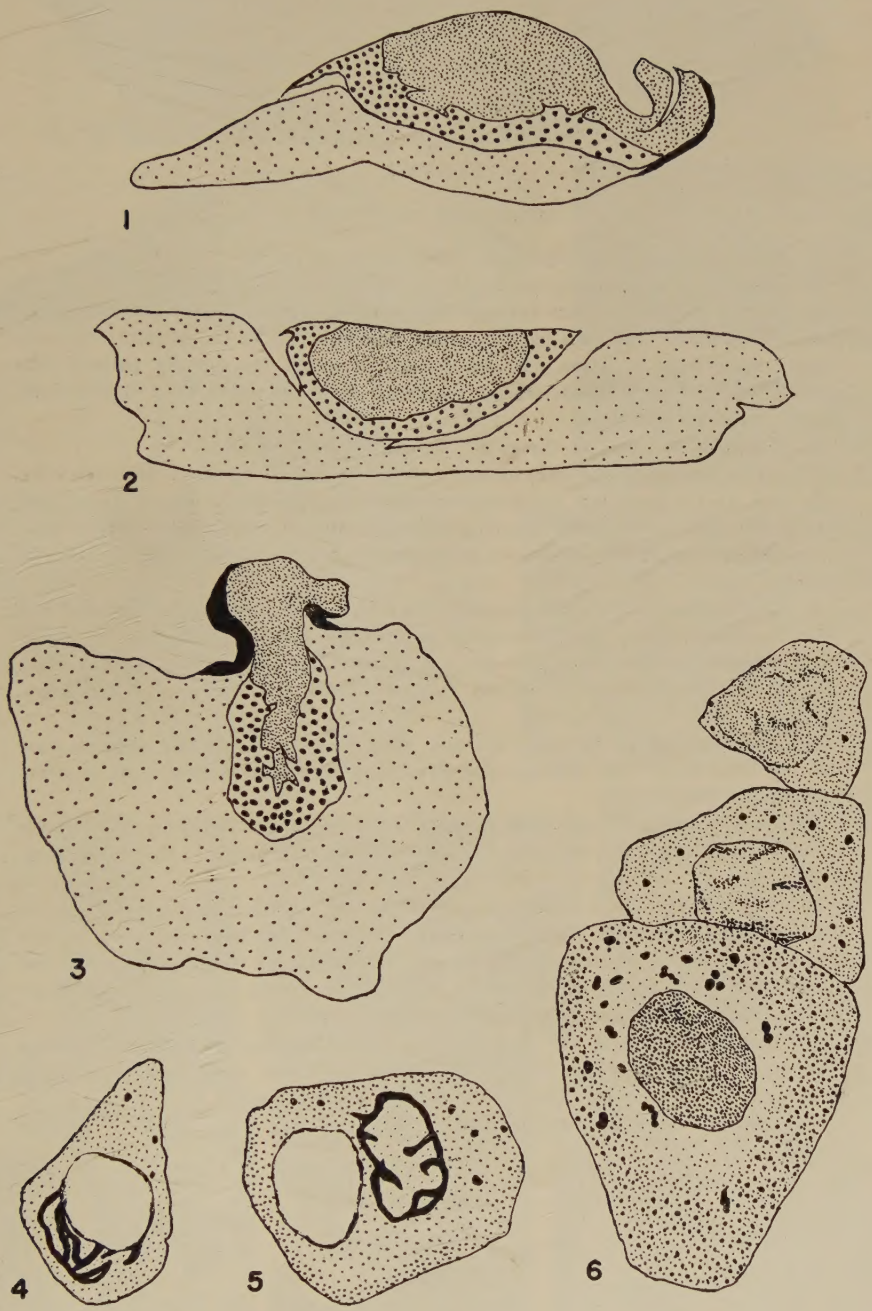
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PLATE 2

EXPLANATION OF FIGURES

7 Description same as figure 2. Helly; Masson. × 40.

8 Description same as figure 1. Helly; Masson. × 36.

9 Median sagittal section through the anterior region of the pituitary of a 2 month-old male. The pars nervosa is shown uppermost in the photomicrograph. Closely applied to it is the pars intermedia in which the densely packed cells may be noted. The pars anterior lies separated from the above by a visible cleft. Helly; Masson. × 220.

10 Description same as figure 3. Helly; Masson. × 36.

11 Cross section of the pars anterior of the animal in figure 2 demonstrating cell types. In the lower left two prominent basophils appear. A densely granulated round acidophil and a larger angular acidophil can be seen in the upper right. Nuclei of several chromophobes are scattered through the figure. Helly; Masson. × 880.

12 Dorsal view of the gland shown in situ with the brain removed. The pituitary (→) lies deeply set in the sella turcica between the large fifth nerves. × 4.

13 Sagittal section of the pars anterior of a 133 day old castrate male showing vacuolation. Dawson and Friedgood formalin-sublimate; Azocarmine technique. × 880.

14 Cross section of the pars anterior of a 9 to 10 month old normal male showing vacuolated cells. Dawson and Friedgood formalin-sublimate; Azocarmine techique. × 880.

15 Cross section of the pars anterior of a 4⅔ month-old pregnant female showing large degranulated basophils lying along a capillary. Helly; Masson. × 880.

16 Sagittal section of the pars anterior of the hamster in figure 5 demonstrating Golgi material. The larger basophils show the Golgi displaced to one side of the nucleus, whereas in the smaller acidophils the Golgi closely caps the nucleus and tends to extend around it. Champy; modified Nassonov-Kolatschev; Dawson and Friedgood Azocarmine technique. × 960.

17 Sagittal section of the pars anterior of the hamster in figure 5 showing the nature and distribution of the mitochondria. These are observed as black granules which are most clearly seen in the large basophils in the photomicograph. Champy-Kull; Altmann-Masson. × 960.

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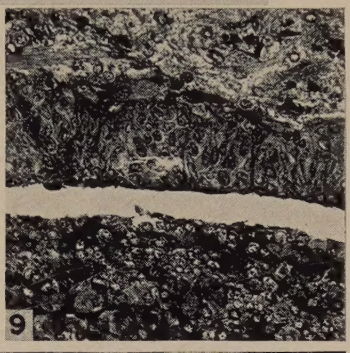
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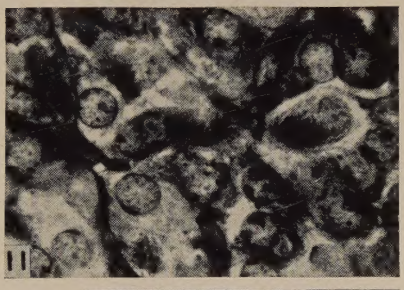
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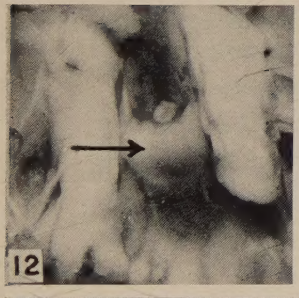
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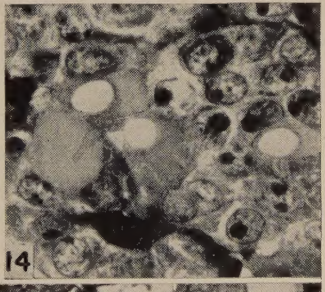
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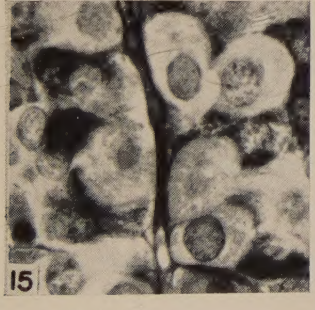
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